CHEPTER 1

INTRODUCTION

1.1 Introduction

The genus *Ceriops* (Rhizophoraceae) comprises two species: *Ceriops decandra* (Griff.) Ding Hou and *Ceriops tagal* (Perr.) C. B. Robinson. They are widely distributed in South and East Africa to Madagascar, Seychelles, Sri Lanka, India, Burma, Andamans, Thailand, Cambodia, Vietnam, southern China, Taiwan, through Malaysia to Micronesia, northern Australia, and Melanesia to New Caledonia. (Duke, 1983). In Thailand, *C. tagal* was found in Chon Buri, Chantaburi, Krabi, Phuket, Satun, Surat Thani and Chumphon. It has local Thai names: Prong Dang (โปรงแดง), Prong (โปรง) and also a synonym of *Ceriops candolleana*, *Ceriops timoriensis*, *Ceriops boiviniana* (Smitinand and Larsen, 1970).

Fruits and hypocotyls

Flowers

Fruits and hypocotyls

Figure 1 The fruits, hypocotyls and flowers of *C. tagal*

Ceriops tagal is an evergreen tree, 5–15(–25) m high and 20–40 cm in diameter, often with unbranched stilt roots and thin knees 20–30 cm high. Bark is light gray or reddish-brown, smooth or irregularly fissured; inner bark is orange or reddish. Leaves are opposite, clustered at end of twigs, obovate to elliptical, 5–10 cm long, 2–6 cm wide, rounded and emarginate at tip, acute at base, entire, thick, leathery, glabrous,

without visible veins. Petiole is 1–3.5 cm long, stipules paired, narrow, ca 2 cm long. Cymes are single and short-stalked in leaf axils. Flowers are 4–10, short stalked, ca 6 mm long. Calyx is yellow-green with 5–6 narrow pointed lobes turned back on fruit; petals 5–6, white, united at base, 2-lobed and ending in 2–4 bristles, stamens 10–12; pistil with conical, partly inferior 3-celled ovary and short style. Berry drooping, ovoid, is 1.5–2.5 cm long, leathery. Seed, viviparous, becoming cigar-shaped or club-shaped, sharply angled, is 15–25(–35) cm long (Duke, 1983).

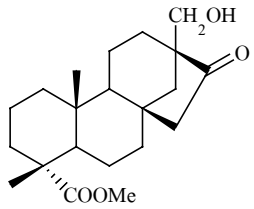
C. tagal has been reported to be used as folk medicine for astringent and hemostat, for treatment of malaria and sores. The shoot decoction, used as a hemostat, has served as a quinine substitute. The bark is also used in lotions for malignant ulcers. Malays give the bark infusion to women in confinement with abdominal ailments. Filipinos used the bark to cure diabetes during the Japanese occupied (Duke, 1983). It is used for the treatment of infected wounds in Thailand and treatment for obstetric and haemorhagic conditions in the Philippines (Bamroongrugsa, 1999).

1.2 Review of Literatures

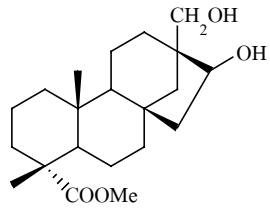
Chemical constituents isolated from the two species of *Ceriops* genus were summarized. Information from NAPRALERT database developed by University of Illinois at Chicaco and SciFinder Scholar copyright in 2006 will be presented.

The triterpenoids (**T1** and **T17-T19**) and steroids (**S1-S5**) were reported by Ghosh et al. (1985) and the investigation from the leaves of *C. decandra*, Ponglimanont and Thongdeeying (2005) resulted in sixteen lupane-type triterpenes (**T1-T16**) and a mixture of oleanolic acid (**T19**) and ursolic acid (**T18**). Ceriopsin A-G (**D1-D7**) diterpenoids isolated from root of *C. decandra* were reported by Anjaneyulu et al. (2002 and 2003). Recently, Zhang et al. (2005) have reported isolation of tagalsin A-J (**D8-D17**), diterpenoids from *C. tagal*. The reported isolates were classified into groups of diterpenoids, triterpenoids, and steroids.

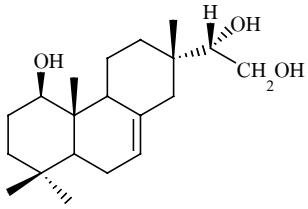
a. Diterpenoids



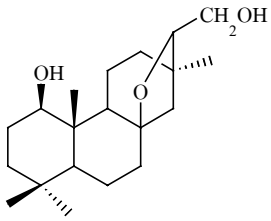
D1: ceriopsin A



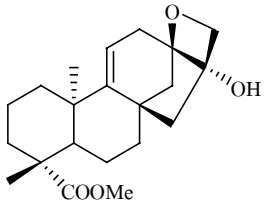
D2: ceriopsin B



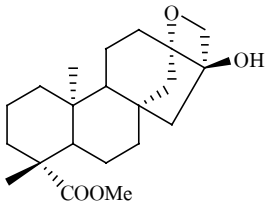
D3: ceriopsin C



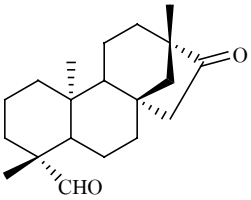
D4: ceriopsin D



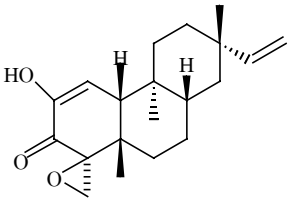
D5: ceriopsin E



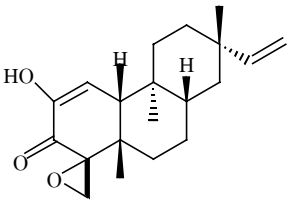
D6: ceriopsin F



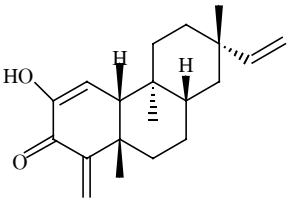
D7: ceriopsin G



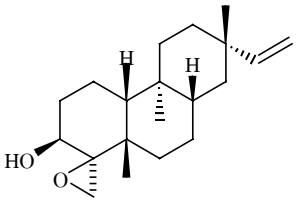
D8: tagalsin A



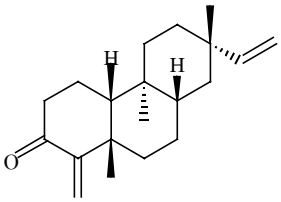
D9: tagalsin B



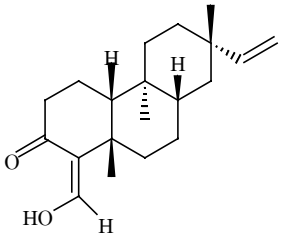
D10: tagalsin C



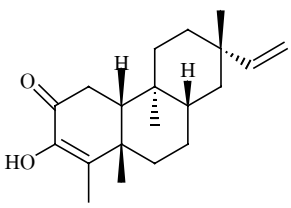
D11: tagalsin D



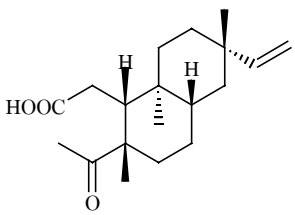
D12: tagalsin E



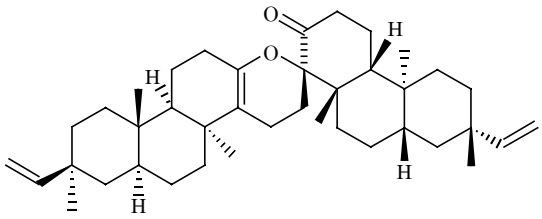
D13: tagalsin F



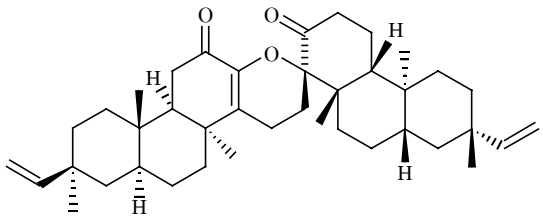
D14: tagalsin G



D15: tagalsin H

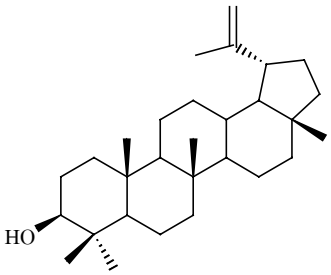


D16: tagalsin I

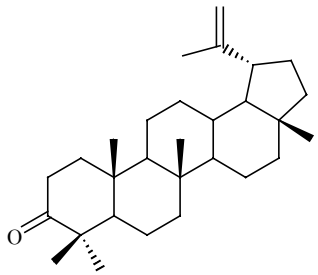


D17: tagalsin J

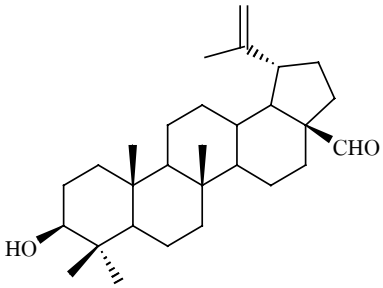
b. Triterpenoids



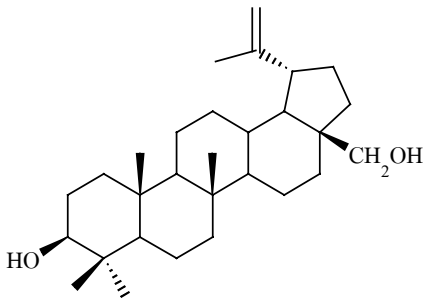
T1: lupeol



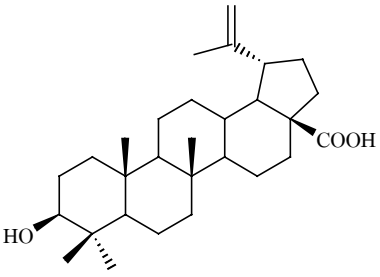
T2: lupenone



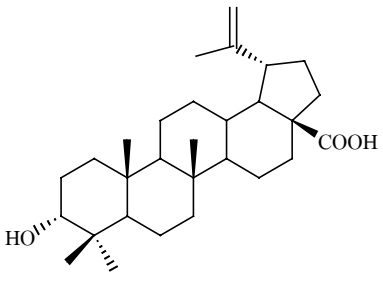
T3: betulinaldehyde



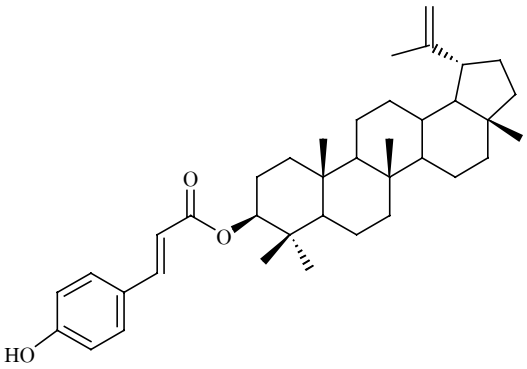
T4: betulin



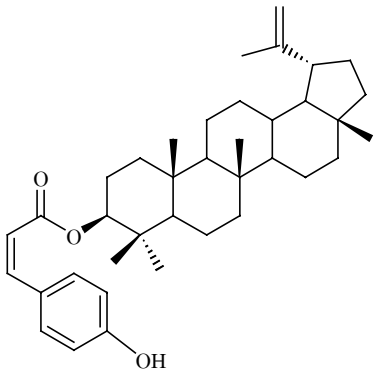
T5: betulinic acid



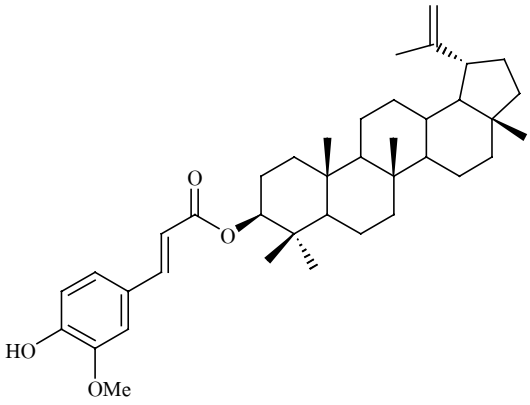
T6: 3-*epi*-betulinic acid



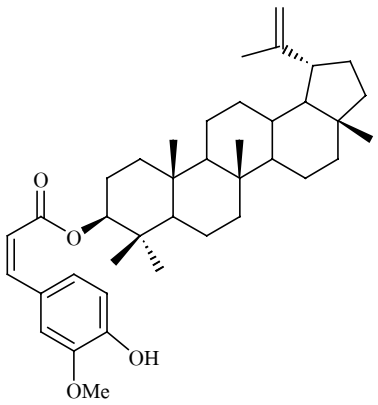
T7: 3β-*E*-coumaroyllupeol



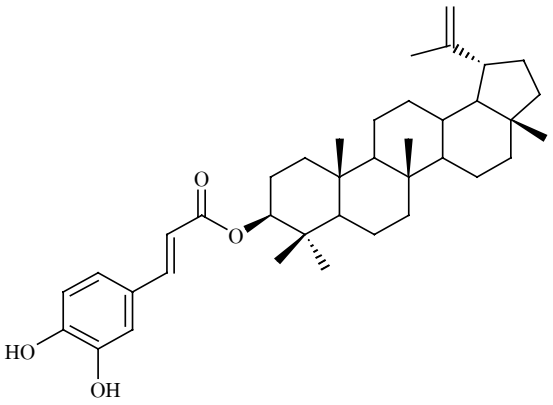
T8: 3β-*Z*-coumaroyllupeol



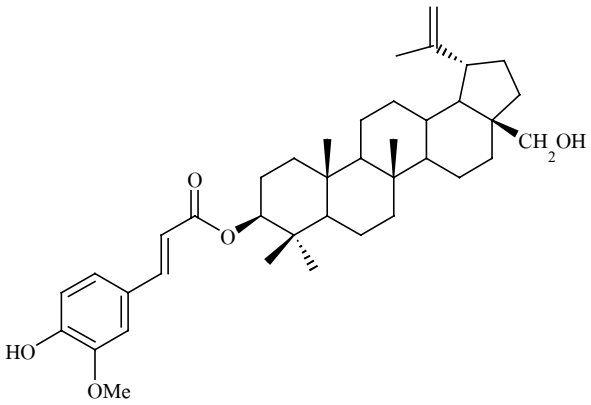
T9: 3β-*E*-feruloyllupeol



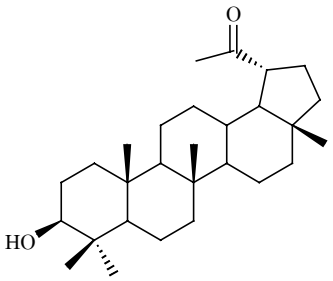
T10: 3β-*Z*-feruloyllupeol



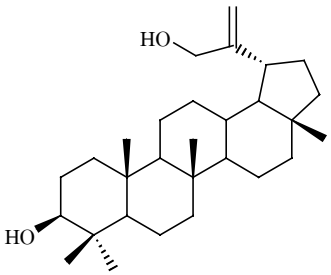
T11: 3β-*E*-caffeoyllupeol



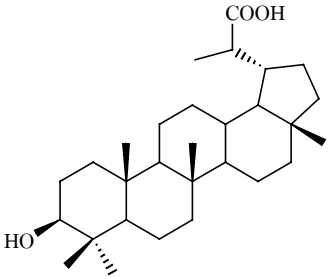
T12: 3β-*E*-feruloylbetulin



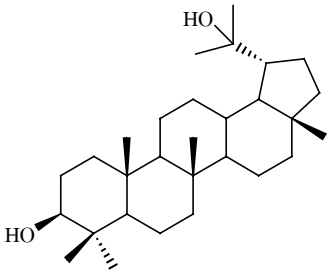
T13: 30-nor-lup-3β-ol-20-one



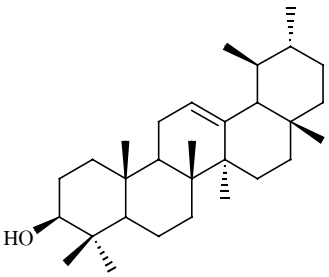
T14: lup-20(29)-ene-3β,30-diol



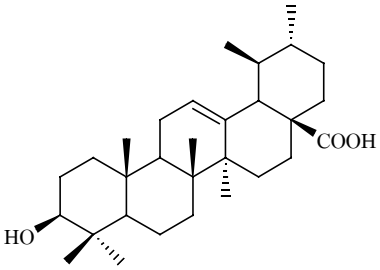
T15: 3β-hydroxylupan-29-oic acid



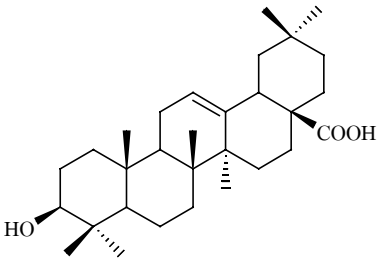
T16: 3β,20-dihydroxylupane



T17: α-amyrin

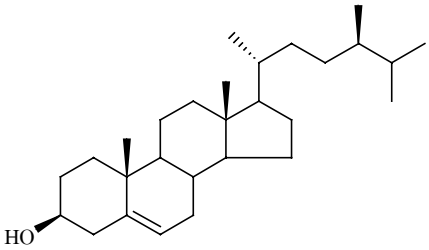


T18: ursolic acid

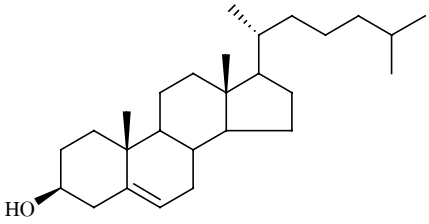


T19: oleanolic acid

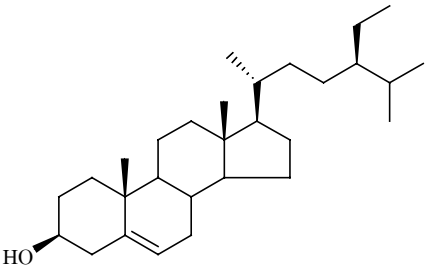
c. Steroids



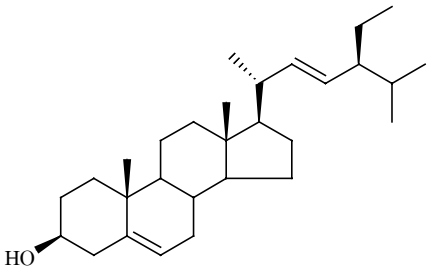
S1: campesterol



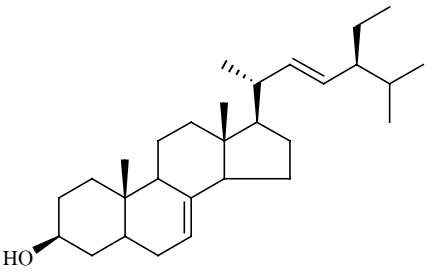
S2: cholesterol



S3: *β*-sitosterol



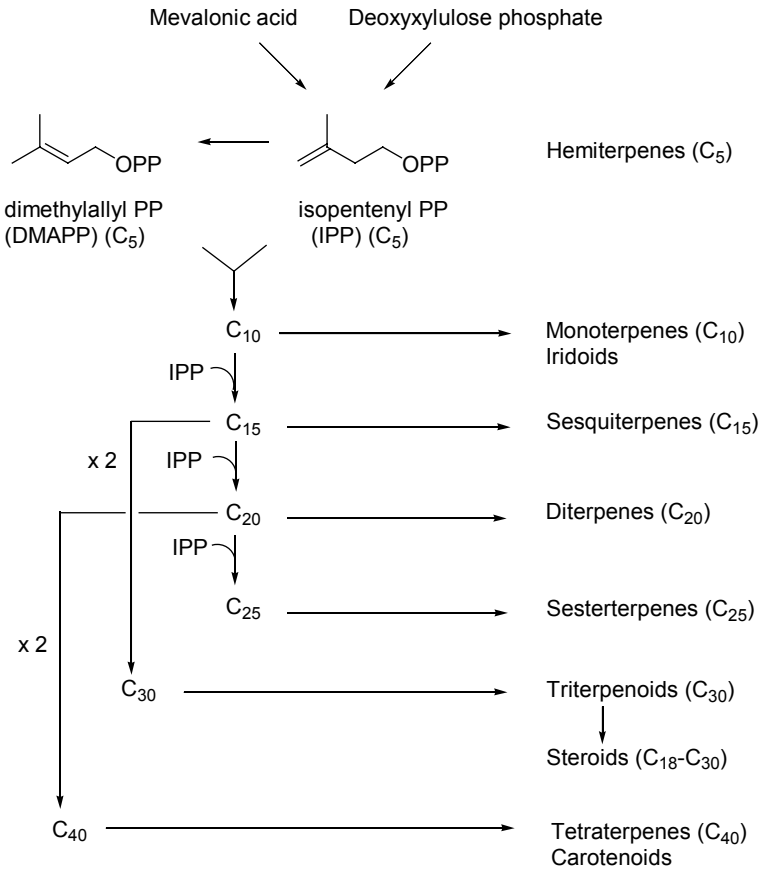
S4: stigmasterol



S5: stigmast-7-en-3*β*-ol

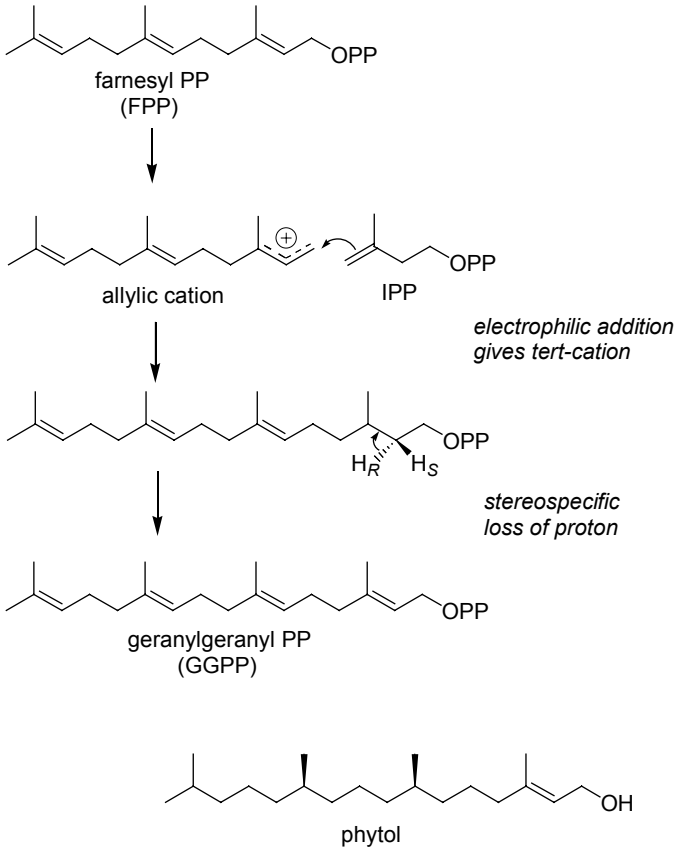
1.3 Biogenesis of pimarane, kaurane, dolabrane, dammarane, lupane and oleanane terpenoids

Terpenoids form a large and structurally diverse family of natural products derived from C_5 isoprene units joined in a head-to-tail fashion. Typical structure contain carbon skeletons represented by $(C_5)_n$, and are classified as hemiterpenes (C-5), monoterpenes (C-10), sesquiterpenes (C-15), diterpenes (C-20), sesterterpenes (C-25), triterpenes (C-30) and tetraterpenes (C-40) (**Scheme 1**). Isoprene itself had been characterized as a decomposition product from various natural cyclic hydrocarbons, and was suggested as the fundamental building block for those compounds, also referred to as 'isoprenoids'. Isoprene is produced naturally but is not involved in the formation of these compounds, and the biologically active isoprene units were identified as diphosphate (pyrophosphate) ester **dimethylallyl diphosphate** (**DMAPP**) and **isopentenyl diphosphate** (**IPP**) (Denwick, 2002).



Scheme 1 Classification of terpenoids $(C_5)_n$ (Denwick, 2002, p 168)

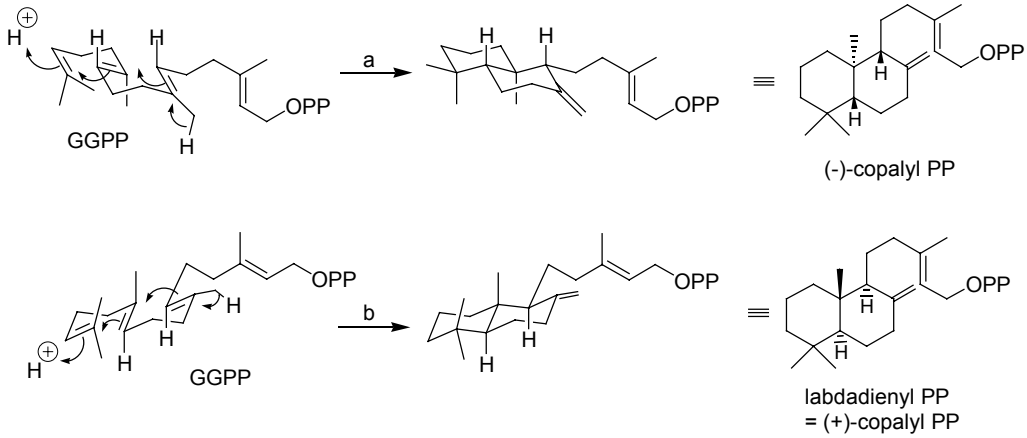
The diterpenes arise from geranylgeranyl diphosphate (GGPP), which is formed by addition of a further IPP molecule to farnesyl diphosphate (**Scheme 2**). One of the simplest and most important of the diterpenes is **phytol**, a reduced form of geranylgeraneol.



Scheme 2 The formation of geranylgeranyl PP (Denwick, 2002, p 204)

Cyclization reactions of GGPP mediated carbocation formation, plus the potential for Wagner–Meerwein rearrangements, will allow many structural variants of diterpenes to be produced. Protonation of GGPP can initiate a concerted cyclization sequence, terminated by loss of proton from a methyl, yielding **copalyl PP** (**Scheme 3**). The stereochemistry in this product is controlled by the folding of the substrate on the enzyme surface, though an alternative folding can lead to **labdadienyl PP**, the enantiomeric product having opposite configurations at the newly generated chiral centers (**Scheme 3**). From copalyl PP, a sequence of cyclizations and rearrangement, all catalysed by a single

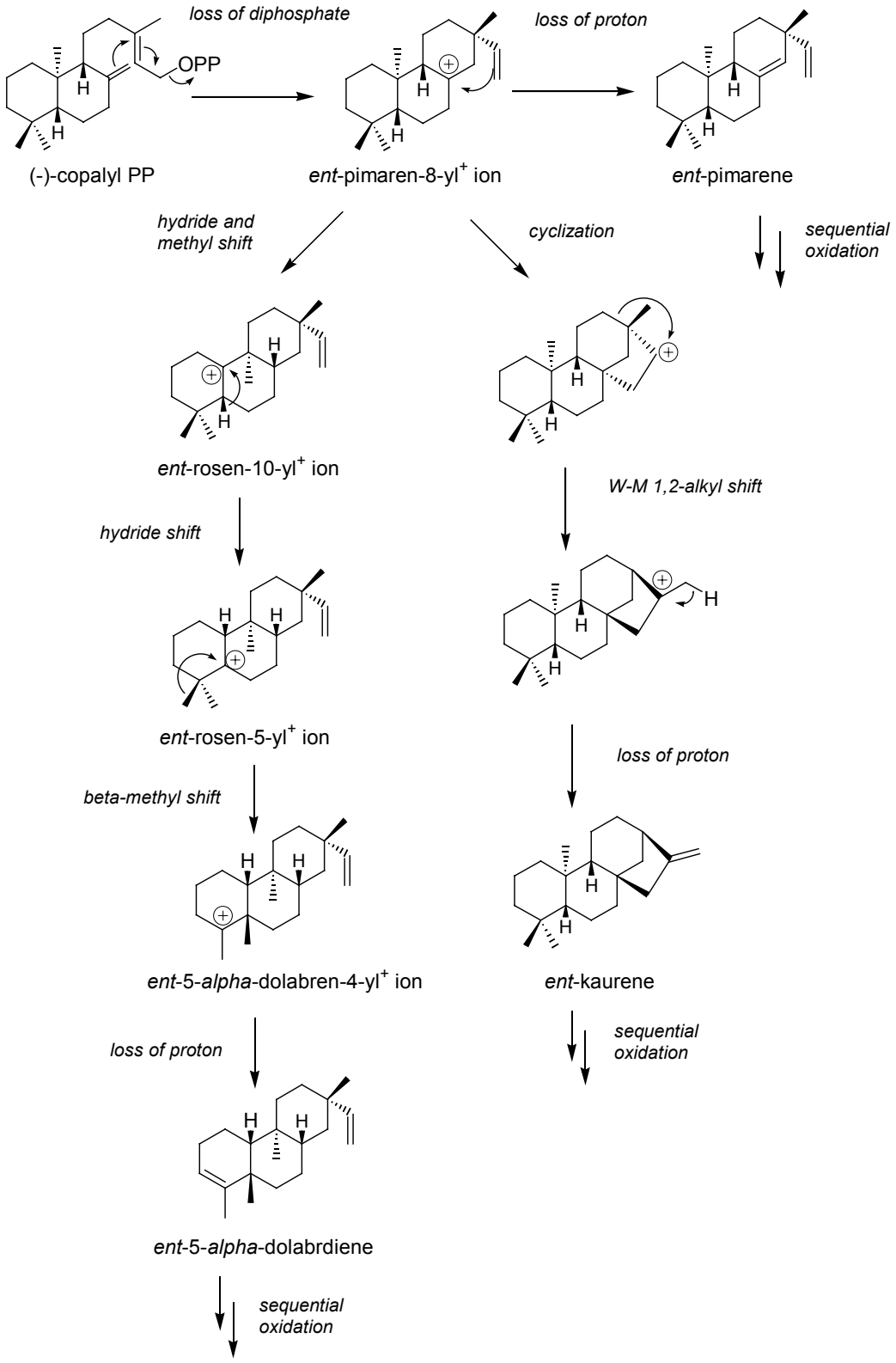
enzyme, lead to *ent*-kaurene (**Scheme 4**). As shown, this involves loss of the diphosphate leaving group enabling carbocation mediated formation of the third ring system, and subsequent production of the fourth ring. Then follows by a Wagner-Meerwein migration, effectively contracting the original six-membered ring to a five-membered one, whilst expanding the five-membered ring to give a six-membered ring. The driving force is transformation of a secondary carbocation to give a tertiary one, but this also results in the methyl group no longer being at a bridgehead, and what appears at first glance to be merely a confusing change in stereochemistry. Loss of a proton from this methyl generates the exocyclic double bond of *ent*-kaurene and provides an exit from the carbocationic system.



Scheme 3 The formation of (-)-copalyl PP and labdadienyl PP (Denwick, 2002, p 208)

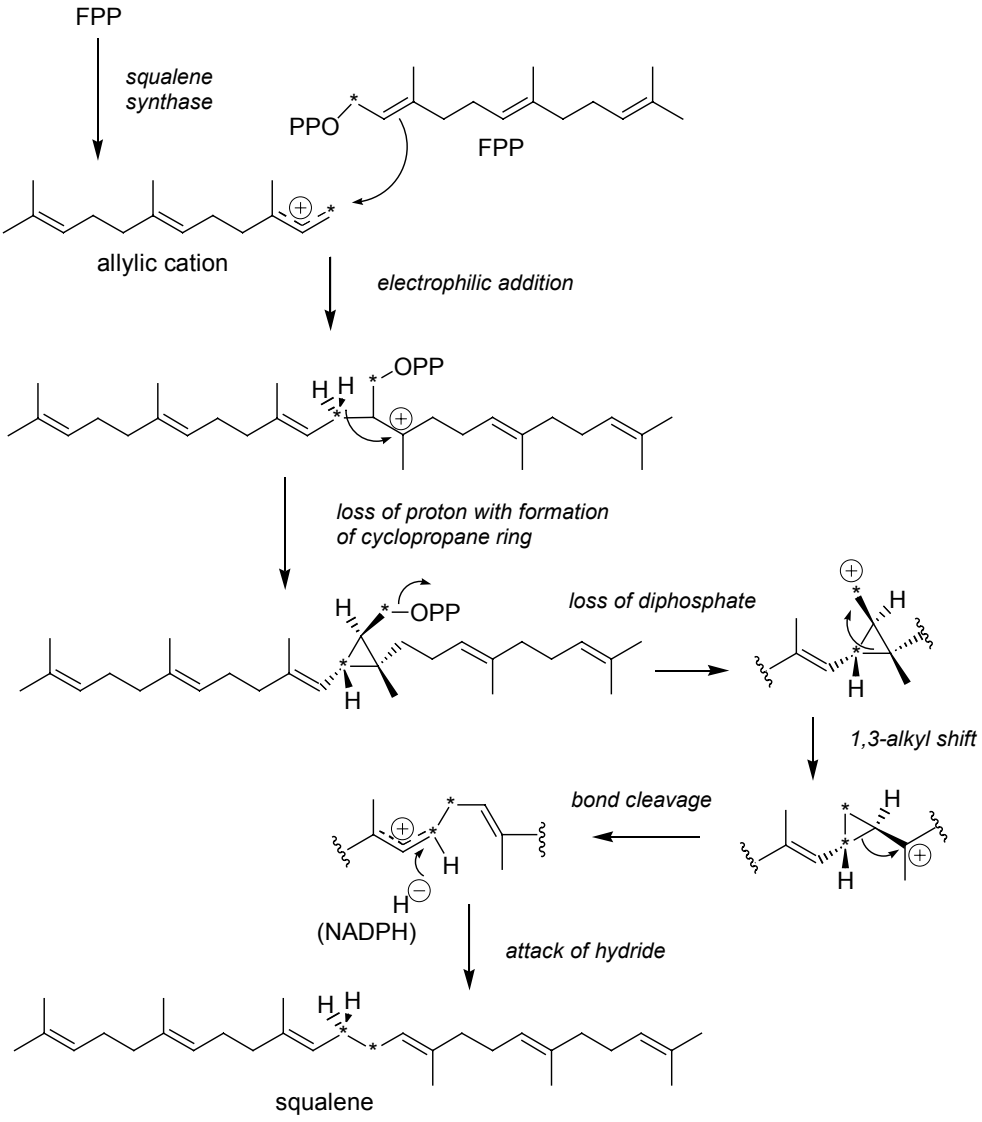
The *ent*-pimarene diterpenes are probably biosynthesized from *ent*-pimaren-8-yl cation (**Scheme 4**).

The *ent*-5α-dolabrenes are likely biosynthesized from *ent*-5α-dolabren-4-yl+ ion precursor that is formed by successive 9→8 hydride, and 10→9 methyl shifts to rosen-10-yl and followed by 5→10 hydride, and 4→5 β-methyl shifts, which have a *cis* AB ring fusion of dolabrane backbone (Grace, 2005), as found in tagalsins from *C. tagal* (**Scheme 4**).

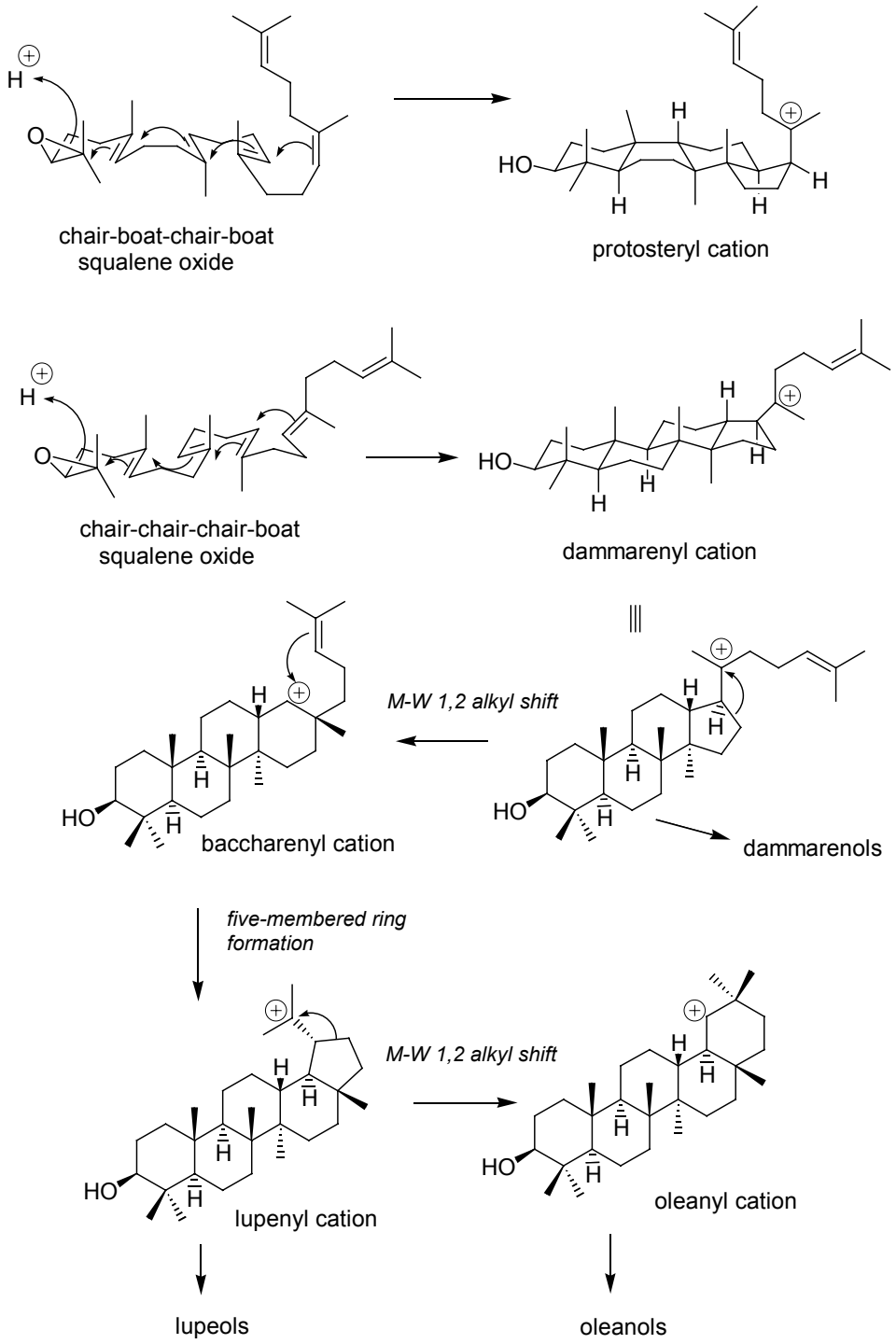


Scheme 4 Biosynthesis of *ent*-pimarenes, *ent*-5α-dolabrenes and *ent*-kaurenes (Denwick, 2002, p 209 and Grace, 2005)

The triterpenes are not formed by an extension of the process of adding IPP to the growing chain. Instead, two molecules of farnesyl PP are joined tail to tail yielding the hydrocarbon **squalene** (**Scheme 5**). Cyclization of squalene is via the intermediate **squalene-2,3-oxide** (oxidosqualene), produced in a reaction catalysed by a flavoprotein requiring O_2 and NADPH cofactors. If squalene oxide is suitably positioned and folded on the enzyme surface, the polycyclic triterpene structures formed can be rationalized in term of series of cyclizations, followed by a sequence of concerted Wagner-Meerwein migrations of methyls and hydrides (Denwick, 2002).



Scheme 5 Formation of squalene (Denwick, 2002, p 214)



Scheme 6 Biosynthesis of dammarenols, lupeols and oleanols (Denwick, 2002, p 216)

The cyclizations are carbocation mediated and proceed in a stepwise sequence. The stereochemistries in cation are controlled by the type of folding achieved on the enzyme surface, and this probably also limits the extent of cyclization process. Thus, if

the folded squalene oxide approximates to a chair-boat-chair-boat conformation, the transient **protosteryl cation** will be produced with these conformational characteristics (**Scheme 6**). Should squalene oxide be folded on to another type of cyclase enzyme, this time in a roughly chair-chair-chair-boat conformation, then an identical carbocation mechanism ensues, and the transient **dammarenyl cation** formed now has different stereochemical features to the protosteryl cation. Should the Wagner-Meewein rearrangements not occur, the dammarenyl cation could be quenched with water, giving the **epimeric dammarenediol.** Alternatively, the migration shown to give the **baccharenyl cation** relieves some ring strain by creating a six-membered ring, despite sacrificing a tertiary carbocation for a secondary one. A pentacyclic ring system can now be formed by cyclization on to the double bond, giving a new five-membered ring and a **lupenyl cation.** Although this appears to contradict the reasoning used above for the **dammarenyl→ baccharenyl** transformation, the condition of the enzyme involved must also be considered in each case. A five-membered ring is not highly strained as evidenced by all the examples encountered. Loss of a proton from the lupenyl cation gives **lupeol.** Ring expansion in the lupenyl cation by bond migration gives the **oleanyl** system. The cyclizations and Wagner-Meerwein rearrangements appear to be catalyzed by a single enzyme, which converts squalene oxide into the final product, e.g. dammarenols, lupeols, or oleanols (**Scheme 6**).

1.4 Objective

The objective of this research is as follow:

- to isolate pure compounds from fruits, hypocotyls and bark of *C. tagal.*
- to determine the structure of pure compounds.
- to evaluate the biological activities of pure compounds such as antimalaria and cytotoxicity.