# CHAPTER II

# LITERATURE REVIEW

## THE GENUS XYLIA

### Taxa and Description

The common name of *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I.C.Nielsen or *X. kerrii* Craib & Hutch. is "Iron wood" and its Thai name is "Daeng". The genus *Xylia* belongs to family Mimosaceae. This genus contains 12 species mostly found in Africa and Madagascar. Only one species has been identified in South East Asia. The species may be divided into two morphological varieties as follows: (เต็ม สมิตินันท์, 2544)

1. *X. xylocarpa* (Roxb.) Taub. var. *xylocarpa* ไคว Khwai (Mae Hong Son), แดง Daeng (General)

Distribution: India and Burmar (Sittiwong, 2003)

2. *X. xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I.C.Nielsen กร้อม Krom (Nakhon Ratchasima), คว้าย Khwai (Chiang Mai, Kanchanaburi), จาลาน Cha-lan (Mae Hong Son), ตะกร้อม Ta-krom (Chunthaburi), ปราน Pran (Surin), ไปรน์ Prai (Si Sa Ket), ผ้าน Phan (Chiang Mai) เพ้ย Phoei (Tak), แดง Daeng (General)

Distribution: Burma, Laos, Cambodia, Vietnam, Thailand (Sittiwong, 2003)

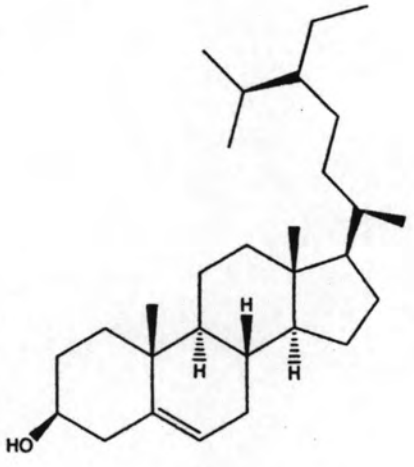
These two varieties are similar but the *xylocarpa* variety has almost glabrous leaflets and anthers with glands, while *kerrii* variety has hairy leaflets and anthers without glands. (สมาคมป่าไม้แห่งประเทศไทย, กรมป่าไม้, 2527) In Thailand, the *kerrii* variety is distributed throughout many regions of country (Sittiwong, 2003). They are deciduous trees, in dry areas usually up to about 25 m tall; old trees on moist fertile sites occasionally up to 37 m., usually with a straight clear bole, sometimes branched from the base. The bark is grey to red with small lenticels, often pitted, peeling off into irregular fragments and occasionally comes out in papery flakes. Branches pointing

upwards, slender, cylindrical, with bipinnate leaves. The leaflet is ovate with an acute tip and oblique leaf base. Leaves spirally arranged, bi-pinnately compound with 1 pair of pinnae, each with 4-5 pairs of opposite oval leaflets, largest terminal pair of leaflets up to 7-20 cm long and 3-7 cm wide. Leaflets oblong-lanceolate, basal ovate, tip bluntly pointed, base rounded or narrow, margin entire, wavy. The tree flowers during March and April at the time of leaf emergence, producing small, pale yellow, round puffs of flowers. 1.4 cm across. Sepal usually 5, always united, like bell-shape. Petal 5, slightly fused at base. Stamen 10 free. Anthers without glands. Pods are grey brown colored, flat, hard, smooth, pointed at tip, narrow at base, 7-10 cm long. Each pod normally contains 5-10 seeds. The seed is flat, brown colored. (สมาคมป่าไม้แห่งประเทศไทย, กรมป่าไม้, 2527)

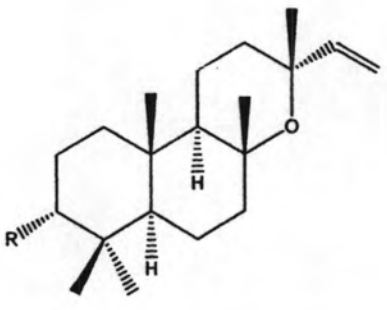
**The Chemistry of the genus *Xylia***

In 1951, Kudzin *et al.* reported the presence of lignin in the heartwood of *X. dolabriformis*.

In 1963, Laidlaw and Morgan reported that chemical constituents isolated from sawdust of *X. dolabriformis* as β-sitosterol **(1)** and 6 diterpenes such as manoyl oxide **(2)**, 3-oxomanoyl oxide **(3)**, sandaracopimaradiene **(4)**, sandaracopimaradiene-3-one **(5)**, sandaracopimaradiene-3β-ol **(6)** and sandaracopimaradiene-3β,18-diol **(7)**. The structures of these compounds are exhibited below.

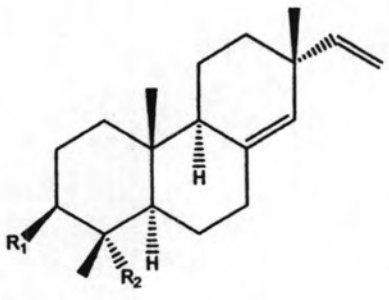



**(1)**




**(2) R = H**

**(3) R = O**




**(4) $R_1 = R_2 = H$**

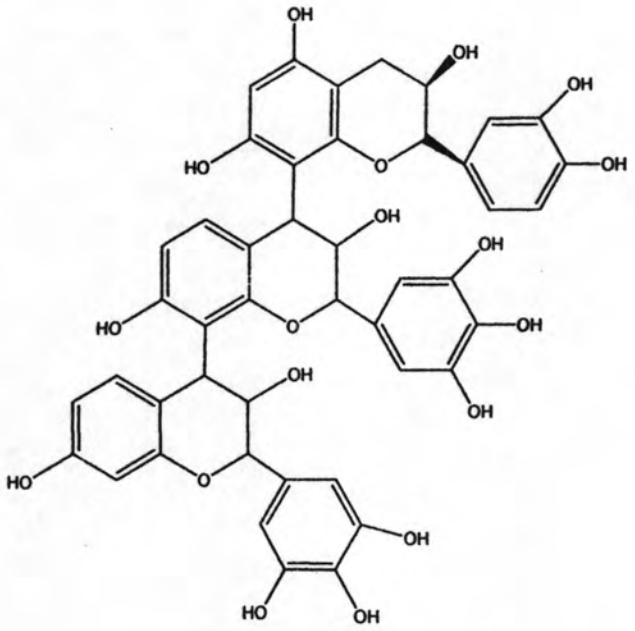
**(5) $R_1 = O$, $R_2 = H$**

**(6) $R_1 = OH$, $R_2 = H$**

**(7) $R_1 = OH$, $R_2 = OH$**

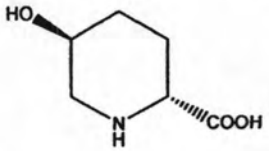
**(7) $R_1 = OH$, $R_2 = CH_2OH$**

In 1976, Kumar *et al.* isolated dolabriproanthocyanidin from the stem bark of *X. dolabriformis*.



**dolabriproanthocyanidin**

In 1979, Master *et al.* isolated trans-5-hydroxy pipecolic acid from the leaves of *X. dolabriformis*.



**trans-5-hydroxy pipecolic acid**

In 1995, Siddhuraju *et al.* reported that the mature seeds of *X. xylocarpa* (Roxb.) Taub. contained 29.5% crude protein, 14.78% crude fat, 8.02% crude fibre, 5.11% ash and 42.6% crude carbohydrates. The seeds appeared to be a good source of potassium, magnesium, phosphorus and iron.

In 2003, Wuntanee Sittiwong isolated eight pure compounds and two mixtures from dichloromethane crude extract of *X. xylocarpa* Taub. These compounds were identified by spectral analysis as 8(14),15-isopimaradiene (sandaracopimaradiene), 8(14),15-isopimaradiene-3-one (sandaracopimaradiene-3-one), 8(14),15-isopimaradiene-

3β-ol (sandaracopimaradiene-3β-ol), 8(14),15-isopimaradiene-3α-ol (sandaracopimara diene-3α-ol), 8(14),15-isopimaradiene-3,18-diol (sandaracopimaradiene-3β,18-diol), 8(14),15- isopimaradiene-18-oic acid (sandaracopimaric acid), 3-oxomanoyl oxide and β-sitosterol. In addition, two mixtures were isolated: a mixture containing 8(14),15-iso pimaradiene-3-one and 7,15-isopimaradiene-3-one and the other one containing 8(14),15-isopimaradiene-3β-ol, 7,15-isopimaradiene-3β-ol and 8(14),15-isopimara-diene-18-ol.

**Biological activities of genus *Xylia***

*Trans*-5-hydroxy pipecolic acid from the leaves of *X. dolabriformis* is reported to be a powerful inhibitor of platelet aggregation induced by serotonin (Master *et al.*, 1979).

50 % Ethanolic extracts from the fruits and bark of *X. dolabriformis* exhibited toxicity ($LC_{50}$ = 510 and 825 mg/kg, respectively) when tested in mice by injection into the abdominal cavity (Aswal *et al.*, 1984).

Dichloromethane crude extract and 8(14),15-isopimaradiene-18-oic acid from the heartwood of *X. xylocarpa* var. *kerrii* exhibited high antifeedant activity against the common cutworm, *Spodoptera litura* and low phytotoxicity against lettuce seedings (Sittiwong, 2003).

## THE PIMARANES

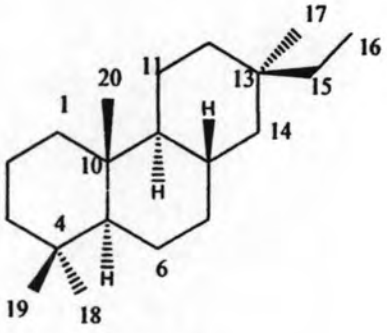
Pimaranes are classified as tricyclic diterpenoids that usually have three fused six-membered rings and are formed from bicyclic diterpenoids by further cyclization (made possible by the loss of the diphosphate). (Pinto *et al.*, 1996, Meragelman *et al.*, 2003)

Diterpenoids are a group of structurally diverse isoprene 4 units or C20 terpenoid compounds that constitute the most abundant and structurally diverse group of plant secondary metabolites, playing an important role in plant-insect, plant-pathogen, and plant-plant interactions. (Cheng *et al.*, 2007). They are commonly present in higher plants and more than 23,000 individual structures have been identified. (Köllner *et al.*, 2004) and are all derived from 2E,6E,10E geranylgeranyl pyrophosphate (GGPP) by a series of biotransformations. They constitute the active constituents of a number of medicinal plants and are of current interest for their potential as future drugs. These compounds are particularly abundant in the Lamiaceae and Asteraceae families, with over 1,200 compounds and found in higher plant, fungi, insect, marine organisms. (Bruneton, 1999)

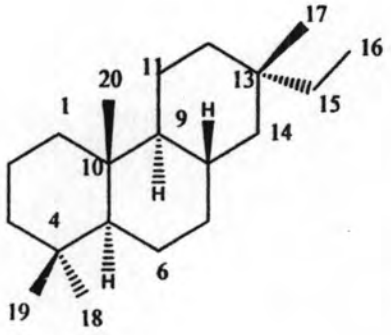
The structures of diterpenes are highly variable and they are classified in accordance with their biogenetic origin. There are two basic groups, acyclic and cyclic diterpenoids. Acyclic diterpenoids are linear and may have cyclic or lactone moieties. Cyclic diterpenoids are classified according to the number of rings (bi-, tri- and tetracyclic). These cyclic diterpenes are further classified in two distinct enantiomeric groups referred to as the *normal* and *ent*-series, with opposite configurations at C-5,C-9 and C-10 (Dewick, 2002).

The pimaranes skeleton has been found in 14 genera. All the pimaranes isolated from *Nepeta* and *Salvia* have a double bond between C-15 and C-16. Additional double bonds are found in *Nepeta* pimaranes between carbons 7(8), 8(9) or 8(14). In these substances the equatorial methyl group (C-18) is bound to an oxygen. The substances from both genera are not oxidized at C-17, C-20 and C-19 (axial methyl at C-4). The compounds found in *Salvia* preserve positions 1, 2, 5, 11 and 12 and in *Nepeta* in 2, 3, 5, 6, 11 and 12 (Vestri *et al.*, 2001).

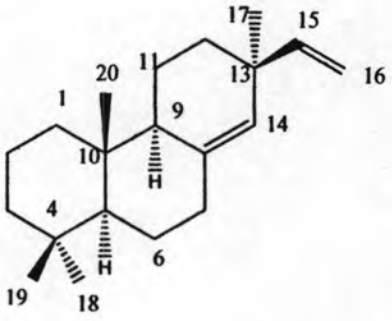
The pimaranes and isopimaranes (formerly called sandaracopimarane) arise by further cyclization of the labdane skeleton. Pimaranes have the ethyl group at C-13 *syn*- to the methyl group at C-10 whereas in the isopimaranes they are *anti*. Both pimaranes and isopimaranes occur in both enantiomeric series (Alhazimi *et al.*, 1994, Fujita, *et al.*, 1984). These structures are commonly found as dienes and the position of the double bond is determined by which proton is removed and they are also defined by the configuration of their biosynthetic precursors into the *ent*- and *normal* series (Dewick, 2002).



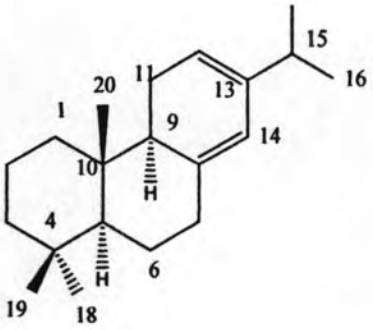

**pimarane**



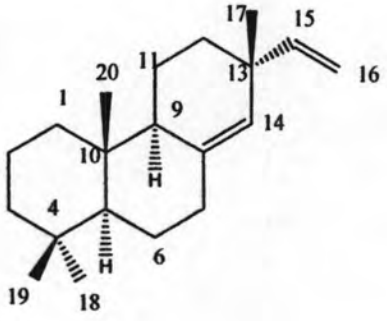

**isopimarane**




**pimaradiene**

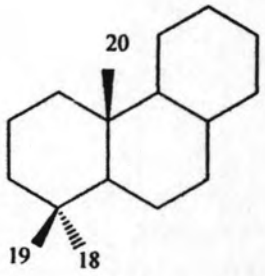



**levopimaradiene**

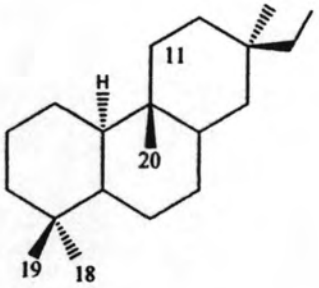



**isopimaradiene (sandaracopimaradiene)**

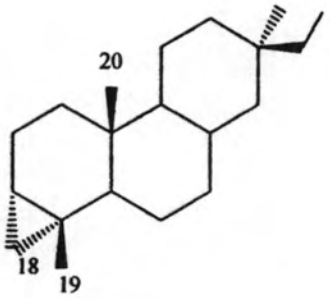
Rearranged and cyclized pimarane include the rosanes (shift of the methyl group C-20 from C-10 to C-9) the parguaranes (3,18-cyclopimaranes), the erythroxylanes (shift of the methyl group C-19 of rosane from C-4 to C-5), and the devadaranes (4,19-cyclo erythroxylanes). Podocarpanes are formally derived from pimaranes by omitting the carbon atoms 15-17 (15, 16, 17-trinorpimaranes) (Breitmaier, 2006).



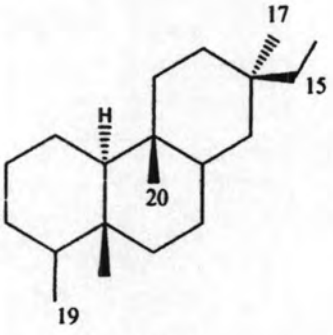

**podocarpane**



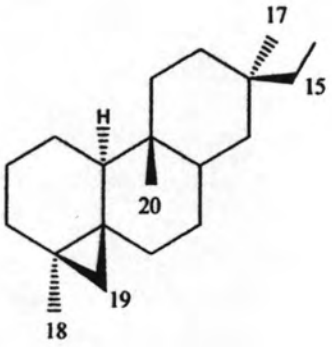

**rosane**




**parguarane**



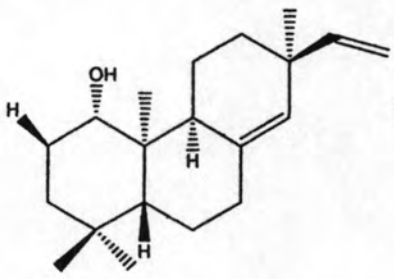

**erythroxylane**



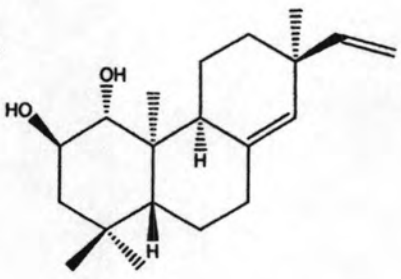

**devadarane**

Structures of naturally occurring pimarane diterpenoids have been reported, for example.

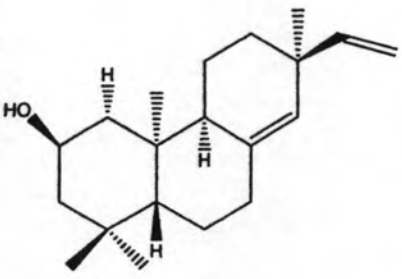
Three *ent*-isopimarane-type diterpenoids were isolated from the New Zealand liverwort *Trichocolea mollissima*, 1α-hydroxy-*ent*-sandaracopimara-8(14),15-diene, (1R, 2R)-*ent*-1,2-dihydroxyisopimara-8(14),15-diene and (2R)-*ent*-2-hydroxyisopimara-8(14), 15-diene.(Cai *et al*, 2003)




**1α-hydroxy-*ent*-sandaraco pimara-8(14),15-diene**

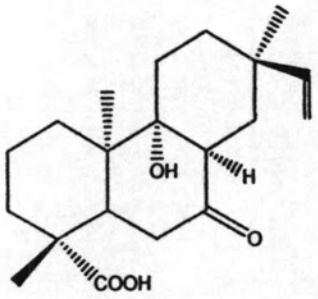



**(1R, 2R)-*ent*-1,2-dihydroxy isopimara- 8(14),15-diene**

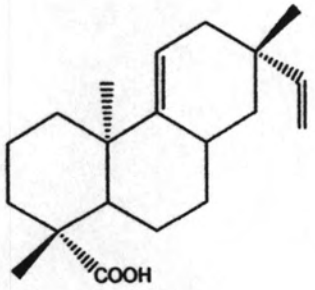



**(2R)-*ent*-2-hydroxyisopimara-8(14), 15-diene**

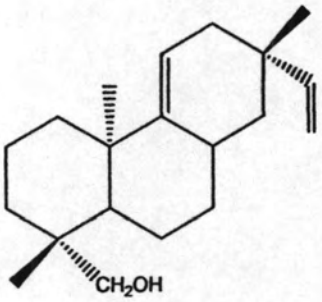
The four pimarane diterpenoids; acanthokorric acid A, acanthoic acid, acanthol and sumogaside were isolated from a $CH_2Cl_2$ fraction of *Acanthopanax koreanum* (Araliaceae) (Cai *et al*, 2003)



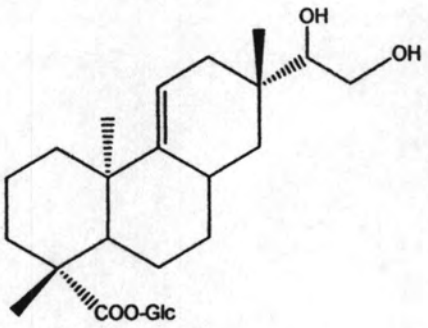

**acanthokorric acid A**




**acanthoic acid**

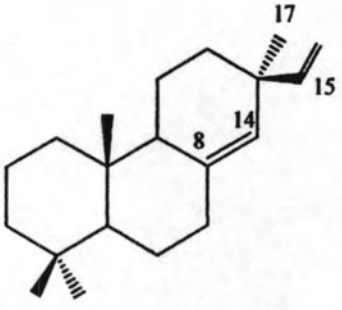



**acanthol**

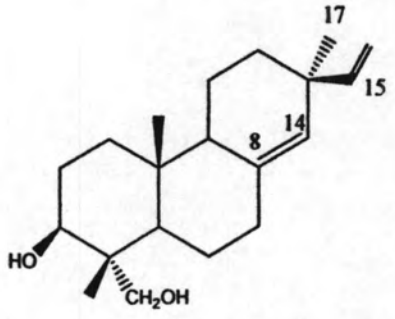



**sumogaside**

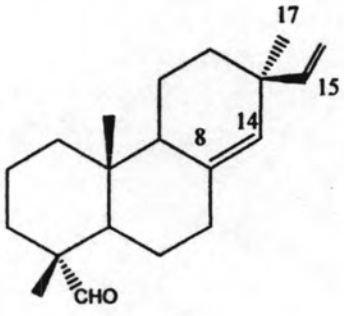
*Pinus silvestris* (Pinaceae), which are wide-spread in Europe, containing pimarane derivatives,for example; (+)8(14),15-pimaradiene-3β,18-diol, 8(14) and 15-pimaradiene-18-al also referred to as cryptopinone, and pimaric acid, isolated from American rosin and belonging to the resin acids of turpentine. The parent 8(14),15-pimaradiene is found among the constituents of *Erythroxylon monogynum* and *Aralia racemosa* (Araliaceae). (Breitmaier, 2006)



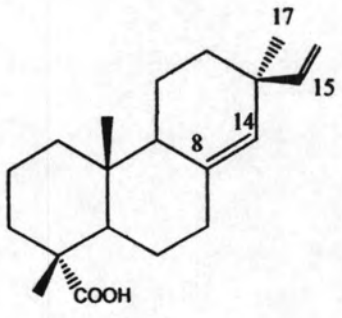

**(+)-8(14),15-pimaradiene**




**(+)-8(14),15-pimaradiene-3β,18-diol**

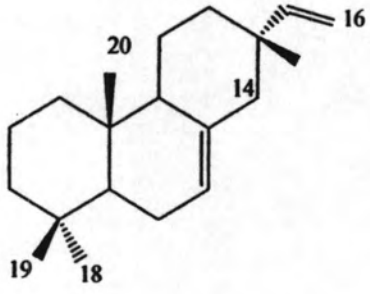



**(+)-8(14),15-pimaradiene-18-al**

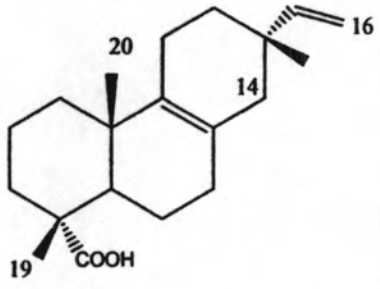



**(+)-8(14),15-pimaradiene-18-oic acid**

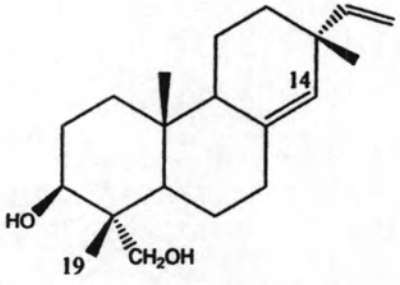
Isopimaranes occur in some pine (Pinaceae) and juniper species (Cupressaceae), for example, 7,15-isopimaradiene, 8,15-isopimaradiene-18-oic acid from *Pinus silvestris* as well as 8(14),15-isopimaradiene-3,18-diol and 8,15-pimaradiene-3,7,19-triol from *Juniperus thurifera* (Breitmaier, 2006).



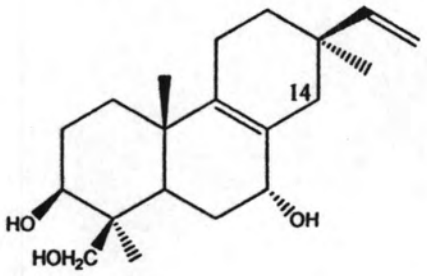

**(+)-7,15-isopimaradiene**




**(+)-8,15-isopimaradiene-18-oic acid**




**(+)-8(14),15-isopimaradiene-3,18-diol**

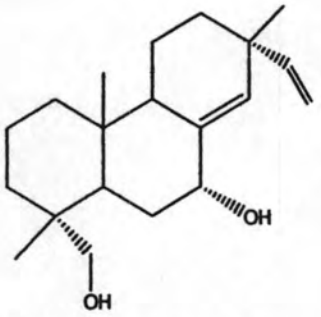



**(+)-8,15-pimaradiene-3,7,19-triol**

**Biological properties of Pimaranes**

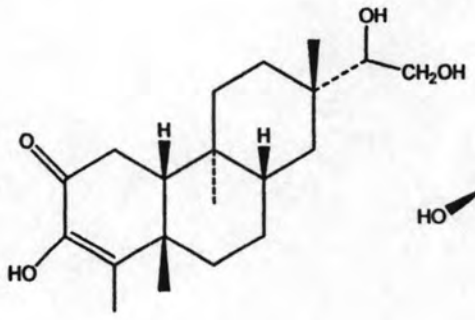
The isolated compound from *Iboza riparia* was 8(14),15-sandaracopimaradiene-7α,18-diol which displayed significant antimicrobial and antispasmodic properties. (Kimpe *et al*, 1982)

8(14),15-Sandaracopimaradiene-7α,18-diol from leaves of *Tetradenia riparia* exhibited significant antimicrobial activity against several bacteria and fungi. (Puyvelde *et al*., 1986)

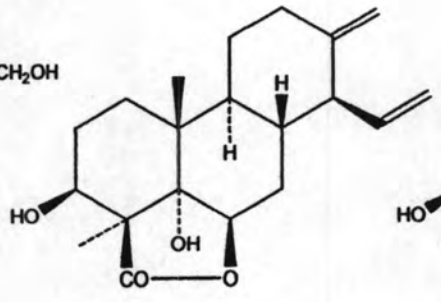



**8(14),15-sandaracopimaradiene-7α,18-diol**

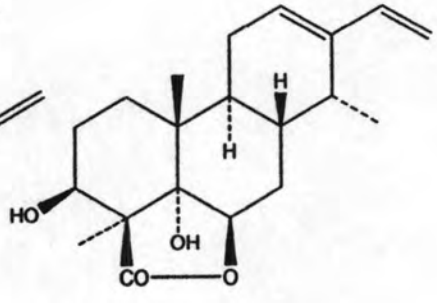
Sandaracopimaric acid has been identified as the lipooxygenase inhibitor from *Juniperus phoenicea.* Extraction of the bark of *Endospermum diadenum* (Euphorbiaceae) afforded the dolabrane and some relatives. A large number of diterpenoid phytoalexins have been detected in rice *(Oryza sativa)* infected with various fungi. The continuing search for novel antifungal agents has afforded the sonomolides A and B, which were obtained from an unidentified coprophilous fungus. (Hanson, 1995)




**dolabrane**



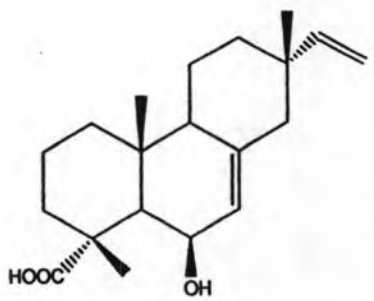

**sonomolides A**



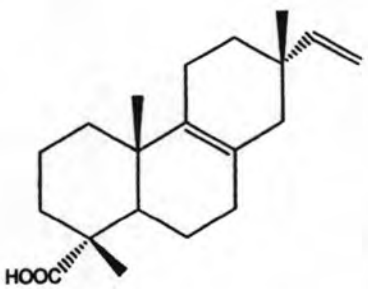

**sonomolides B**

Acanthoic acid from the $CH_2Cl_2$-soluble fraction of *Acanthopanax. Koreanum* showed potent inhibitory activity on the IL-8 secretion of the TNF-α-stimulated human colon adenocarcinoma cell line HT-29 and on the TNF-α secretion of the trypsin-stimulated human leukemic mast cell line HMC-1 which are found at high level in the colon lumen of patients with ulcerative colitis (Cai *et al*, 2003).

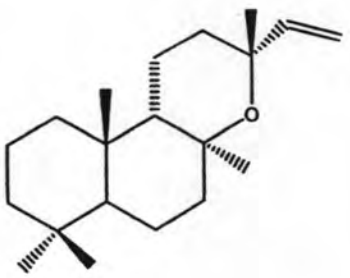
The pimarane diterpenoid 6β-hydroxyisopimaric acid, from *Salvia caespitosa*, displayed strong activity against *S. aureus, S. epidermidis and B. subtilis,* while sandaracopimaric acid was found to be active only against *S. aureus* and *B. subtilis*. Sandaracopimaric acid from *Juniperus excelsa*, showed moderate activity against *Candida albicans*. The pimarane diterpenoid 13-epimanoyl oxide was isolated from Turkish *Sideritis perforiata*, and manoyl oxide were tested against *S. aureus, S. epidermidis, S. hominis, K. pneumoniae* and *E. coli*. The 13-*epi* isomer was found to be active against staphylococci, but it and other manoyl oxide mixtures were inactive against gram negative bacteria. A new pimarane, salvipimarone diterpenoid was isolated from *Salvia multicaulis,* and showed activity against *P. mirabilis* and *E. faecalis*. It also showed strong antituberculosis activity against *Mycobacterium tuberculosis* strain H37Rv with the following an MIC value of 7.3 μg/ml. (Topçu and Gören, 2007)



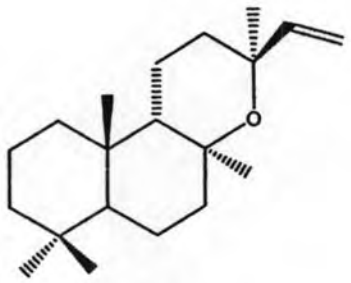

**6β-hydroxyisopimaric acid**



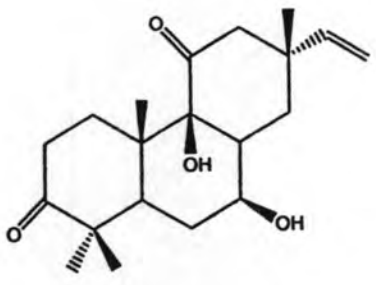

**sandaracopimaric acid**




**manoyl oxide**

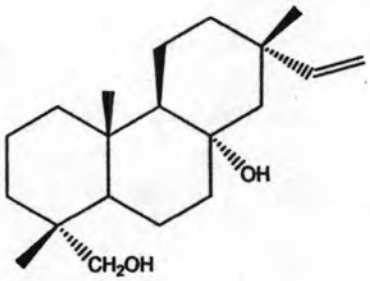



**13-epimanoyl oxide**

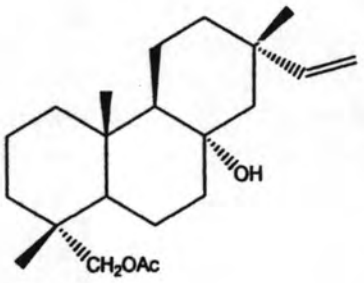



**salvipimarone**

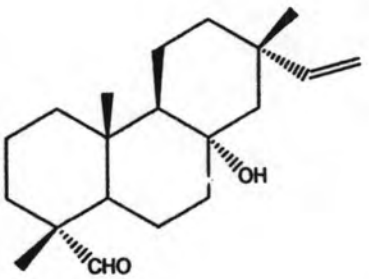
Three pimarane diterpenoids from leaves and wood of *Tetraclinis articulate*; 13-*epi*-Pimar-16-ene-8α,18-diol, 8α-hydroxy-13-*epi*-pimar-16-en-18-yl acetate and 8α-hydroxy-13-*epi*-pimar-16-en-18-al were reported at 10 μM significantly inhibited certain human leukocytes functions such as the degranulation process measured as myeloperoxidase and elastase release, and the superoxide production measured by chemiluminescence. These phenomena are involved in a large number of pathophysiological functions, and their modulation is considered an interesting strategy in the control of inflammatory disorders. (Barrero, *et al.*,2003)




**13-*epi*-Pimar-16-ene-8α,18-diol**

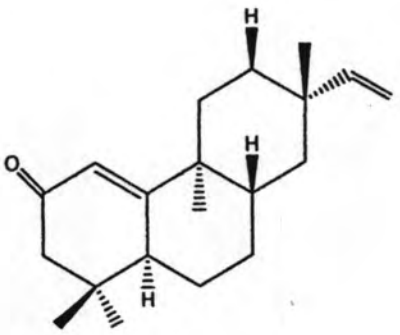



**8α-hydroxy-13-*epi*-pimar-16-en-18-yl acetate**



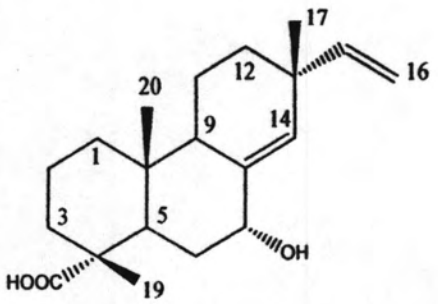

**8α-hydroxy-13-*epi*-pimar-16-en-18-al**

Petalostigmones from extraction of *Petalostigma pubescens* heartwood exhibited cytotoxic against mouse leukemia cell lines and mouse liver cancer.




**petalostigmones**

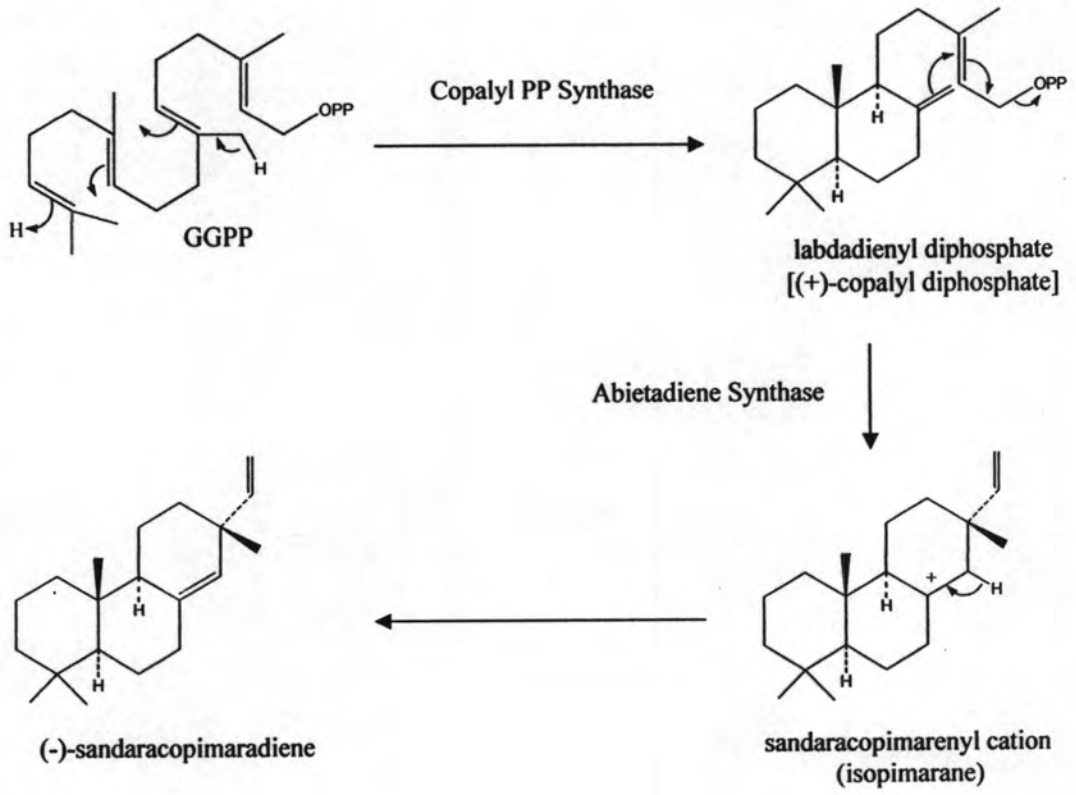
7α-Hydroxysandaracopimaric acid was isolated from the leaves of *Juniperus taxifolia* induced cellular differentiation in a concentration range of 0.39-6.29 μM and may also cause apoptotic cell death at the higher concentrations. These results may provide a basis for the potential therapeutic application of this compound and its related compounds to cancer therapy (Muto *et al.*, 2008).




**7α-hydroxysandaracopimaric acid**

**Biosynthesis of Pimaranes**

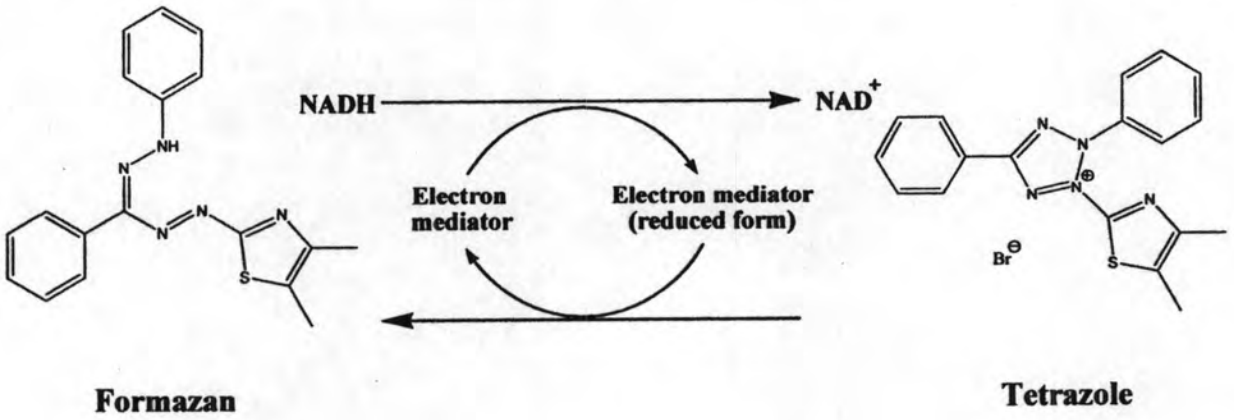
Pimarane diterpenes arise by further cyclization of (+)-copalyl or *ent*-copalyl PP. Loss of the PP allows for carbocation mediated formation of the third ring systems, and is accompanied by Wagner-Meerwein shifts. Cyclization of (+)-copalyl (labdadienyl) is catalyzed by abietadiene synthase and can occur by attack on C-13 by the exocyclic double bond or by the migration of the C-13 double bond and stabilization of the cation by elimination of a proton from C-7 or C-14. This sequence of events results in the formation of isopimaradienes. (Dewick, 2002)




**Scheme 1 Biosynthesis of Pimaranes**

## CYTOTOXICITY

Cytotoxic test was carried out at the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University. Bioassay of activity against human cancer cell culture *in vitro* was performed by the MTT (3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) colorimetric method. Yellow a tetrazole is reduced to purple formazan in the human cancer cell line. In principle, the viable cell number/well is directly proportional to the purple formazan product. A dimethyl sulfoxide (DMSO), solubilization solution is added to dissolve the insoluble this product into a colored solution. The absorbance of this colored solution can be quantified by measuring at 540 nm wavelength spectrophotometer. When the amount of purple formazan produced by human cancer cell lines treated with an agent is compared with the amount of formazan produced by untreated control human cancer cell lines, the effectiveness of the agent in causing death of human cancer cell lines can be increased, through the production of a dose-response curve. (Mosmann, 1983, Wilson, 2000, Vol.1)




**Formazan** **Tetrazole**

**Scheme 2 MTT reduction**