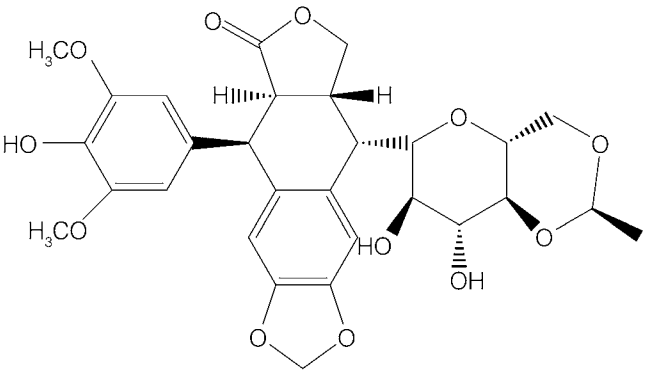
# 3 Plants for Chemotherapy of Neoplastic Diseases

## GENERAL CONCEPT

Each year in the United States more than 1 million people are diagnosed with cancer, and about 500,000 people die from the disease. For the most part, the reason that cancer is a fatal disease is that cancer cells can invade through, and metastasize to, distant organs in the body. The hallmarks of malignant neoplastic tissue are unregulated cell proliferation, invasiveness, and metastasis to distant sites in the body. Surgery and radiotherapy can eradicate localized tumors but may fail because the cancer may have metastasized to other areas of the body; chemotherapy, if used properly, may control or eliminate metastasis. The array of drugs used for the treatment of cancer includes antimetabolites (methotrexate [Trexall®]), fluoouracil (Efudex®), mercaptopurine (Puri-Nethol®), cytarabine (Cytosar®), covalent DNA-binding drugs (nitrogen mustards, alkylating agents), noncovalent binding drugs (anthracyclines), antiestrogens, and inhibitors of chromatin function.




Etoposide (Vepesid®)

Examples of inhibitors of chromatin function derived from flowering plants (Fig. 80) are etoposide (lignan) and alkaloids camptothecin, *Vinca* alkaloids, and 7 epitaxol. The rhizome of *Podophyllum peltatum* L. (May apple, Berberidaceae) has been used to remove warts and to relieve the bowels from costiveness since very early times. It contains podophyllotoxin, a cytotoxic lignan from which etoposide (Vepesid®), which is used to treat lung cancer, lymphomas, and leukemias on account of its ability to inhibit the activity of

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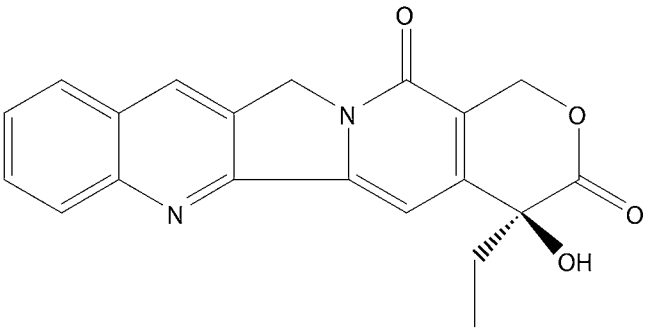
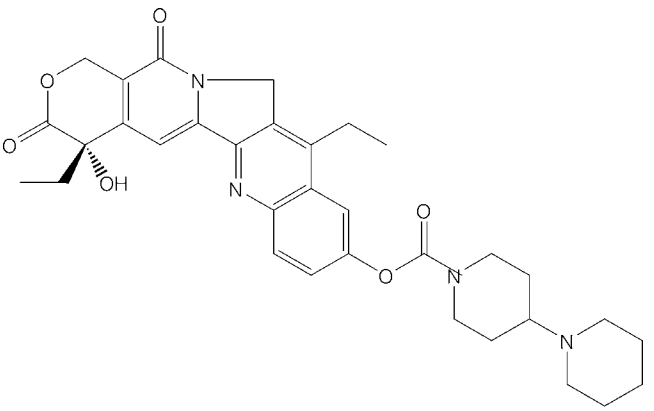
topoisomerase, has been semisynthetically developed Attempts to verify the reputed antidiabetic property of *Catharanthus roseus* G. Don (periwinkle, Apocynaceae) in the 1960s led first to the observation by Canadian workers that leaf extract caused leucopenia in rats.

Researchers from Eli Lilly pharmaceuticals, (a company founded in 1876 by Colonel Eli Lilly veteran of the US Civil War), undertook further intensive phytochemical studies and characterized 60 alkaloids, of which a group of 20 binary indole alkaloids—including vincristine and vinblastine. Vinblastine sulphate (Velbe®) inhibits the polymerization of tubulin and is used to treat generalized Hodgkin's disease and chorionepithelioma, whereas vincristine sulphate (Oncovin®) is used to treat leukemia in children.




Camptothecin

Camptothecin is a monoterpenoid quinoline alkaloid that is also known to occur in the subclass Rosidae: *Camptotheca acuminata* Decsne. (family Nyssaceae, order Cornales), and *Nothapodytes fetida* (Wight.) Sleum. (family Icacinaceae, order Celastrales); and the Asteridae: *Ophiorrhiza mungos* L. (Rubiaceae). Camptothecin was found to inhibit topoisomerase and to be active against experimental tumors; however, initial clinical trials showed little response and severe cystitis, but more effective analogs were developed, such as irinotecan (Campto®). Cancer chemotherapy alone, however, is not very effective in producing long-term survival or treating the most common solid tumors, and the need for new anticancer drugs is critical.




Irinotecan (Campto®)

A possible source for chemotherapeutic agents is the medicinal flora of the Asia–Pacific region. The purpose of this chapter is to provide a fundamental approach to understanding the potential of the medicinal flora of this region as a source of new anticancer drugs.

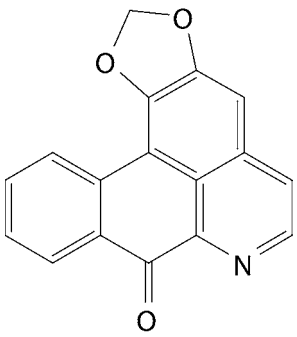
## TOPOISOMERASE INHIBITORS

Topoisomerases temporarily break DNA strands and perform topological changes to selected regions of the genome available for transcription. Two main classes of topoisomerases are recognized to date: topoisomerases I and II. Topoisomerase I catalyzes the ATP-independent relaxation of DNA supercoils by transiently breaking and religating single-stranded DNA. Topoisomerase II relaxes supercoiled DNA through catalysis of a transient breakage of double-stranded DNA in an ATP-dependent manner. Examples of topoisomerase inhibitors are etoposide and camptothecin, which form a stable ternary DNA–topoisomerase II drug complex that maintains a cleaved state of DNA and interferes with DNA replication, repair, and transcription of eukaryotic cells (Fig. 80).

### Medicinal Annonaceae

The evidence for the existence of anticancer agents in the family Annonaceae is strong and it seems likely that further research on this taxa will lead to the discovery of antineoplastic agents. Among the families of flowering plants, Annonaceae are particularly interesting in the field of oncology because this family elaborates a surprisingly broad array of secondary metabolites, which abrogate the survival of mammalian cells, including acetogenins, styryl-lactones, and isoquinoline alkaloids.

Sung et al. made the interesting observation that liriodenine, an aporphine isolated from Cananga odorata inhibited the enzymatic activity of topoisomerase II both in vivo and in vitro and caused highly catenated simian virus 40 daughter chromosomes in simian virus 40-infected CV-1 cells (1). Aporphine alkaloids from Annonaceae, but also the Magnoliidae in general, are likely to have potential as inhibitors of topoisomerase, and further investigation of flowering plants, and especially the medicinal Annonaceae of the Asia–Pacific region, as a source of cytotoxic aporphoids is encouaged.




liriodenine

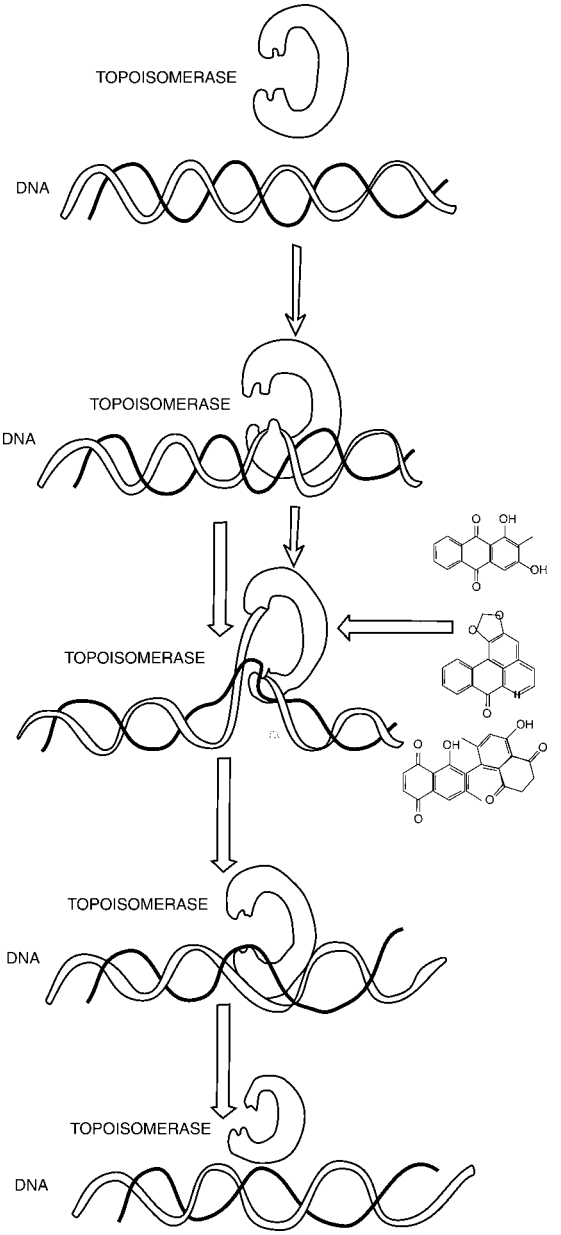
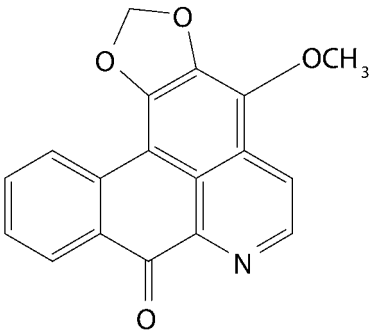



Fig. 80. DNA damage induced by inhibition of topoisomerase II.

***Artabotrys suaveolens* Bl.**, or *akar cenana* (Malay), is a woody climber that grows in the primary rainforest of Burma, Java, Moluccas, and the Philippines. The bark is blackish and smooth, and the twigs are hooked. The leaves are simple, alternate, dark green, glossy, elliptic, and 8.5 × 3.5 cm–5 × 3 cm. The fruits are green, glossy, and ellipsoid (1.4 cm × 5 mm), with ripe carpels, each containing a single seed. Indonesians

drink a decoction of the leaves to treat cholera. In the Philippines, decoction of bark and roots is drunk to promote menses and to relieve childbirth exhaustion.




Atherospermidine

The potential properties of *A. suaveolens* Bl. has a source of topoisomerase II inhibitor is open for exploration. The plant probably elaborates aporphine alkaloids because aporphines are known to occur in the genus *Artabotrys* (*2,3*). Liriodenine and atherospermidine from *Artabotrys uncinatus* and artabotrine from *Artabotrys zeylanicus* abrogated the survival of cancer cells cultured in vitro (*4*).

***Fissistigma fulgens* (Hk. f. et Th.) Merr.** (*Melodorum fulgens* Hk. f. et Th, *Uvaria fulgens* Wall.), or *pisang hutan* (Malay), is a large climber that grows in the primary rain forest of south peninsular Malaysia. The plant is easily recognized by the shining leaves with minute adpressed, tawny pubescence on the lower surface of the leaves. The stems are terete and pubescent. The leaves are simple, alternate, and exstipulate. The blade is oblong lanceolate, 7.5–15 cm × 3–5 cm, acute, the base is rounded. The blade shows 13–18 pairs of secondary nerves running out to the margin. The petiole is 7–10 mm long. The flowers have a slight sweet odor, and are terminal in a few flowered cymes. The flower pedicels are 5–10 mm long. The sepals are broadly ovate, pubescent outside, and 1–2 mm long. The petals are thick and orange; the outer petals are ovate-oblong and 1.2–1.5 cm long, and the inner petals are 7 mm long. The fruits are ripe carpels, 3–4 cm long, and 2.3 cm in diameter (Fig. 81).

In Malaysia, a paste of leaves is applied to sore legs, and a decoction of the leaves is drunk as a protective remedy given after childbirth. To date, the pharmacological potential of *F. fulgens* (Hk. f. et Th.) Merr. is unexplored. It would be interesting to learn whether further study on this medicinal plant disclose any aporphines of chemotherapeutic interest.

***Friesodielsia latifolia* Hk. f. et Th.** (*Oxymitra latifolia* Hk. f. et Th.) is a climber that grows in the primary rain forest of Southeast Asia. The plant was present in Singapore in the Mac Ritchie Reservoir forest and the Botanic Garden's jungle. It is a climber that can grow to a length of 20 m. The young branches are rusty tomentose. The leaves are simple, alternate and exstipulate, and large. The blade is coriaceous,

Fig. 81. *Fissistigma fulgens* (Hk. f. et Th.) Merr. Flora of Malaya. Herbarium Singapore. Field Collector: Mohd. Shah Mahmud. No 4996. Date: 7/21/1984. alt: 1200 feet. Terengganu.

dark green on top, glaucous on the bottom, glabrous (except for the midrib), glossy, broadly obovate, and 18 × 7.5 cm. The blade shows 10 pairs of secondary nerves. The petiole is 1 cm long. The flowers are solitary on 1-cm-long pedicel. The sepals are coriaceous, sub-orbicular, rusty-pubescent, and 4 mm long. The petals are creamy white turning brown, 4 cm long, inner to 2.7 cm long. The fruits are ripe carpels of about 1.5 cm in length and 7 mm diameter, oblong-ovoid, apiculate, and slightly pubescent (Fig. 82).

The plant is used by Malays to assuage body pains, and a decoction of roots is drunk as a protective remedy after childbirth. The pharmacological properties of this plant are unknown, but it is very probable that it elaborates aporphines and flavonoids as characterized in *Oxymitra velutina* (5).

## Medicinal Lauraceae

Taxonomically close to the Annonaceae, the Lauraceae family abounds with aporphinoid alkaloids. A remarkable advance in the search for topoisomerase inhibitors from Lauraceae has been provided by Woo et al. (6). Using DNA-unwinding assay and structural modeling, they showed that dicentrine can attain a relatively planar conformation and molecular bulk which allow it to occupy the active site of topoisomerase II which becomes inactive. The requirement of a suboptimal conformation to achieve DNA binding appears to make dicentrine less potent against topoisomerase II than the

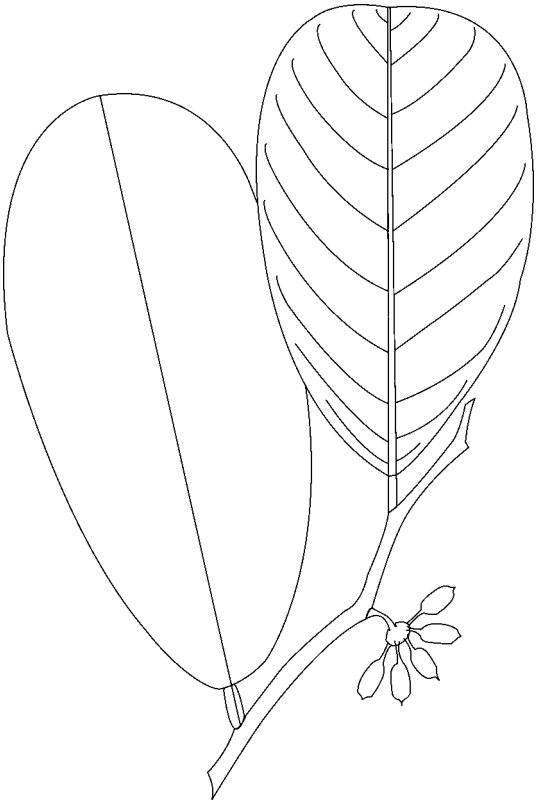
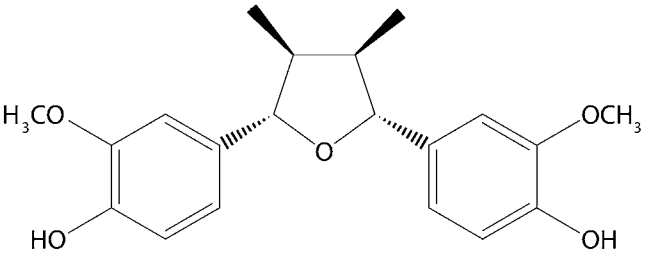


Fig. 82. *Friesodelsia latifolia*. Singapore Field No: 29434. The Botanic Gardens Singapore. 5/14/1935. Field collector: E. Corner. Botanical identification: J. Sinclair, 11/21/1953.

very planar oxoaporphine alkaloid liriodenine (*7*). Other inhibitors of topoisomerase present in Lauraceae are diaryldimethylbutane lignans. Such compounds are found in *Persea thunbergii* (Sieb. & Zucc.) Kosterm.

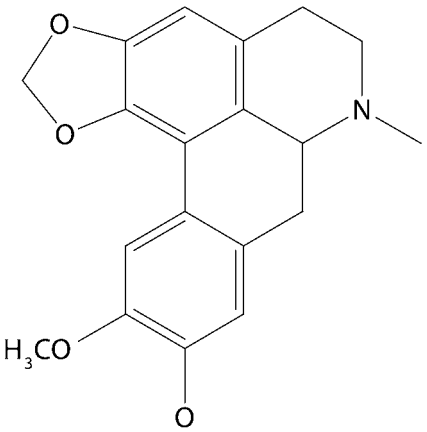
***P. thunbergii* (Sieb. & Zucc.) Kosterm**. (*Machilus thunbergii* Sieb.& Zucc.), or common machilus, *tabunoki* (Japanese), is a tree native to Korea and Japan and is also found in Taiwan. The bark is smooth, fawn, and lenticelled. The leaves are spiral, simple, exstipulate-elliptic, glossy, and somewhat fleshy. In Japan and Korea, the plant affords a remedy for eczema, diseases of the spleen and stomach, and asthma.

Li et al. using bioassay-guided fractionation, isolated a number of diaryldimethylbutane lignans, of which meso-dihydroguaiaretic acid, which inhibited the enzymatic activity of topoisomerase I and II by 93.6 and 82.1%, respectively, and nectandrin B showed 79.1 and 34.3% inhibition, respectively, in vitro at a concentration of 100 mM (*8*). Note that such lignans are present in the Myristicaceae, and one might set the hypothesis that lignans with potent topoisomerase inhibitors await discovery in the Laurales–Magnoliales group.



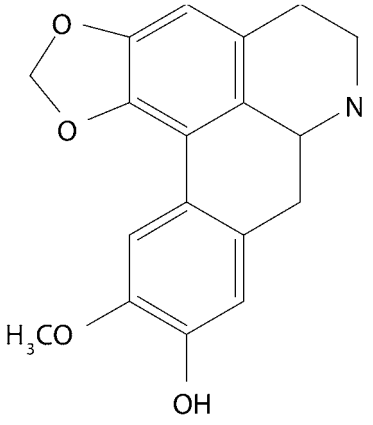

Diaryldimethylbutane lignans

***Lindera tzumu* Hemsl.** (*Sassafras tzumu* [Hemsl.]) Hemsl, *Pseudosassafras tzumu* (Hemsl.) Lecte., *Pseudosassafras laxiflora* (Hemsl.) Nakai, *Lindera camphorata* Levl., or *cha mu, mu wang* (Chinese), is a tree that grows in China. The plant grows to 35 m tall and 2–5 m in diameter. The bark is yellow-green, smooth, and irregularly and longitudinally fissured. The wood is yellowish, finely grained, durable, and used for boat and furniture making. Leaves are simple, alternate, aggregate at apex of stems, and exstipulate. The blade is ovate or obovate and 9–18 × 6–10 cm. The fruits are subglobose, up to 10 mm in diameter, blackish-blue, and seated on a cup-shaped perianth. The wood of this tree is highly esteemed by the Chinese, who believe that a house built of this timber is never struck by lightning. The drug consists of the white inner bark, which is used as an anthelmintic, parasiticidal, antiseptic, anti-emetic, and antipyretic. The pharmacological potential of this plant is unexplored yet. Note that D-dicentrine, from the root of *Lindera megaphylla* Hemsl. abrogated the survival of a number of cancer cell lines cultured in vitro including esophageal carcinoma HCE-6, lymphoma cell lines Molt-4 and CESS, leukemia cell lines HL60 and K562, and hepatoma cell line MS-G2, and significantly inhibited the tumor incidence of leukemia cell line K562 in severe combined immunodeficient mice (9).
The cytotoxic activity of dicentrine is mediated via inhibition of topoisomerase II (6,7). Dicentrinone from *Ocotea leucoxylon* is closely related to dicentrine, and even more planar has shown potent topoisomerase I activity (9).

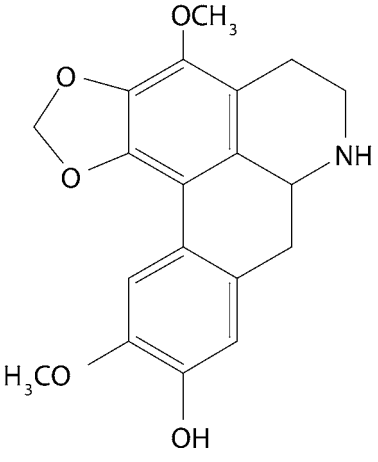



Dicentrine

***Cassytha filiformis* L.**, mentioned earlier, contains aporphine alkaloids such as actinodaphnine, cassythine, and dicentrine, which effectively bind to DNA and behave as typical intercalating agents and interfere with the catalytic activity of topoisomerases (*3,10,11*).



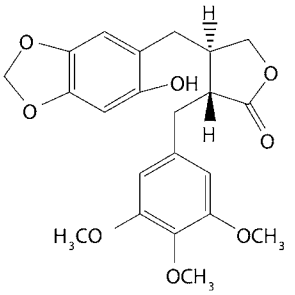

Actinodaphnine



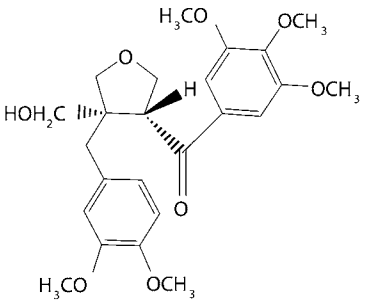

Cassythine

## Medicinal Hernandiaceae

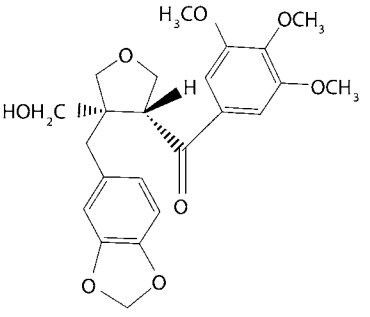
The family Hernandiaceae consists of four genera: *Hernandia*, *Illigera*, *Gyrocarpus*, and *Sparattanthelium*, and about 60 species of trees, shrubs, and woody climbers widespread in tropical regions. An example of Hernandiaceae is *Hernandia ovigera* L., which is grown as a tropical street tree. Hernandiaceae are member of the order Laurales and are known to abound with aporphines and lignans.



16-Hydroxyyatein



Hernone

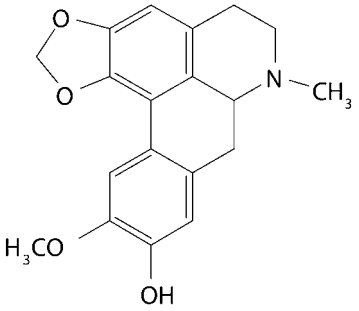


Nymphone

Lignans characterized from the bark of Hernandia nymphaeifolia: (2)-69-hydroxyyatein, (2)-hernone, and (2)-nymphone exhibited some levels of cytotoxic activities against P-388 and HT-29 cell lines with ED50 values of 0.321 and 0.740 mg/mL for (2)-nymphone; and 0.806 and 0.909 mg/mL for (2)-hernone (12). In the Asia–Pacific region, Illigera appendiculata Bl., Illigera luzonensis L., and H. ovigera L. (Hernandia peltata Meissn.) are medicinal.

***Illigera luzonensis* L.** (*Henschelia luzonensis* C. Presl., *I. luzonensis* (C. Presl.) Merr., *Henschelia luzonensis* C. Presl., *Gronovia ternata* Blanco, *Halesia ternata* Blanco, *Illigera meyeniana* Kunth ex Walpers, *Illigera pubescens* Merr., *Iligera ternata* [Blanco] Dunn.), or *tai wan qing teng* (Chinese), is a climber that grows in Taiwan, Japan (Ryuku), and the Philippines. The stems are angular. The leaves are simple, compound, and spiral. The petiole is 4–10 cm long, rough, and hairy. The folioles are ovate, rounded at base, mucronate at apex, and somewhat pubescent. The influorescences are 7–15 cm axillary cymes. The fruits bear a pair of wings.

In the Philippines, the sap expressed from the stem is drunk to alleviate headache. Using antiplatelet aggregation as a guide to fractionation, Chen et al. isolated a series of aporphines including actinodaphnine, *N*-methylactinodaphnine, launobine, dicentrine, *O*-methylbulbocapnine, hernovine, bulbocapnine, and oxoaporphines; dicentrinone and liriodenine were isolated from the stems of *I. luzonensis* (13).

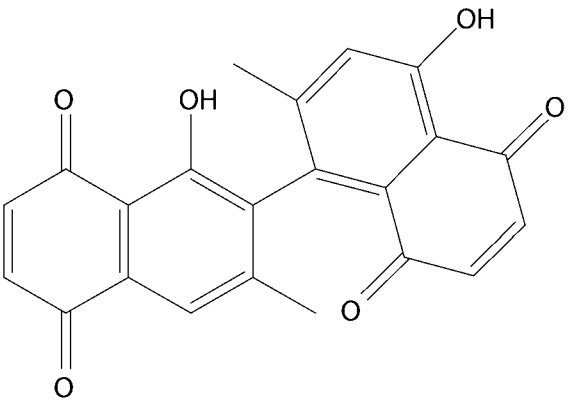



*N*-methyl-actinodaphnine

*N*-methyl-actinodaphnine possesses 5-hydroxytryptamine receptor blocking activity and a selective antagonist of $\alpha_1$-adrenoceptors, selective for the $\alpha_{1B}$- than for the $\alpha_{1A}$-adrenoceptor subtype (*14*). What are the activities of *N*-methyl-actinodaphnine and other aporphines of *Illigera* and *Hernandia* species against topoisomerase?

## Medicinal Ebenaceae

The family Ebenaceae consists of five genera and about 450 species of trees of which 20 species are used for medicinal purposes in the Asia–Pacific region. Ebenaceae and particularly *Diospyros* species have attracted a great deal of interest for their dimers and oligomers of naphthoquinones, which are antibacterial, antiviral, monoamine oxidase inhibitors, and cytotoxic. Ting et al. made the interesting observation that isodiospyrin is cytotoxic via direct binding topoisomerase I, which limits the access of the enzyme to the DNA substrate and prevents both DNA relaxation and kinase activities of topoisomerase I (*15*). Therefore, an interesting development from Ebenaceae would be a systematic investigation of naphthoquinone for topoisomerase inhibition.




Isodiospyrin

Fig. 83. *Diospyros sumatrana.* From Oxford University Department of Forestry. Forest Herbarium. T.D. Pennington. 9/11/1963. No: 7807. From: FRIM Kepong. No: 94504. Det: Ahmad.

***Diospyros sumatrana* Miq.** (*Diospyros flavicans* [Wall.] Hiern, *Diospyros dumosa* King & Gamble, *Diospyros decipiens* King & Gamble, *Diospyros tubicalyx* Ridl., *Diospyros vestita* Bakh., *Diospyros velutinosa* Bakh., *Diospyros hendersoni* Ridley.), is a tree that grows to a height of 30 m tall and a girth of 100 cm in Indonesia, Thailand, and Malaysia (Sabah, Borneo) in lowland rainforest up to 1500 m. The stems are hairy when young. The leaves are simple, elliptic, ovate, oblong, 3.5–20 × 1.2–6.5 cm, the apex is acuminate, the base is pointed, and the midrib sunken above. There are 3–11 pairs of secondary nerves. The male flowers are 5-merous in 3–10 flowered subsessile cymes, and show 16 anthers. The female flowers are four-merous, salver-shaped, and show a four-locular ovary. The fruits are globose, 1.2 × 2.4 cm, with a 2.5-cm diameter calyx (Fig. 83). Malays drink a decoction of roots as a protective remedy. The plant has not been studied for its pharmacological potentials.

***Diospyros toposioides* King & Gamble**, or *arang, kayu arang* (Malay), is a tree that grows to a height of 13 m and a girth of 60 cm in lowland rainforests of Malaysia (Borneo). The leaves are simple, oblong, oblong-ovate, 16–33 × 4–14 cm, the apex is acuminate, the base is rounded, and the midrib is sunken above; the secondary nerves are inconspicuous and loping at margin. The male flowers are in three-flowered axillary cymes and show 35–96 anthers. The female flowers are four-merous and show a eight-locular hairy ovary. The fruits are globose, 5 cm in diameter on a 3-cm-wide calyx (Fig. 84). In

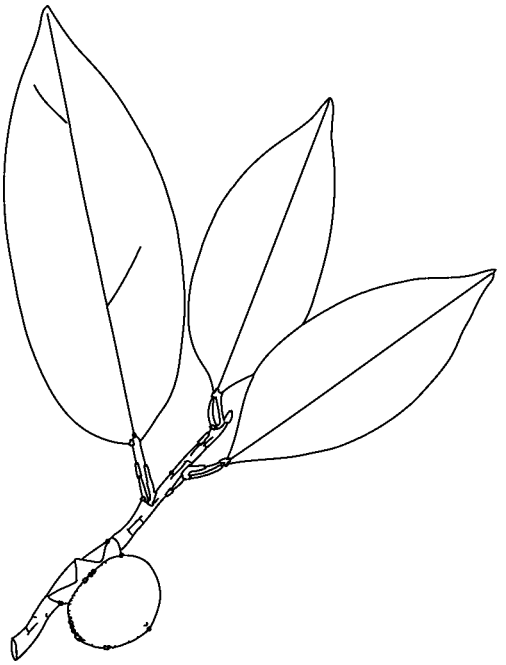
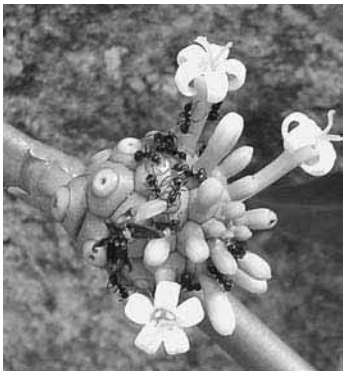


Fig. 84. *Diospyros toposioides.* Flora of Malaya. Field No 2417. Geographical localization: Chior, Perak. Field collector: 7/11/1967. K.M. Kochummen.

Malaysia, the seeds are poisonous and used to catch fish. The plant has not been studied for its pharmacological potentials. The ichthyotoxic property could involve some naphthoquinones and/or saponins.

## Medicinal Rubiaceae

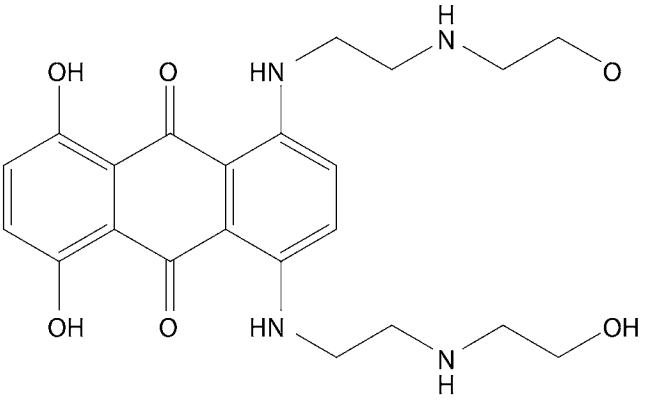


The family Rubiaceae consists of about 450 genera and 6500 species of tropical and subtropical trees, shrubs, climbers, and herbs that are known to abound with iridoid glycosides (monoterpenoid alkaloids, tannins, and anthraquinones). When looking for Rubiaceae in field collection, one is advised to look for plants with opposite simple leaves with an interpetiolar stipule, tubular flowers, which are often white, and capsules, berries, or drupes.

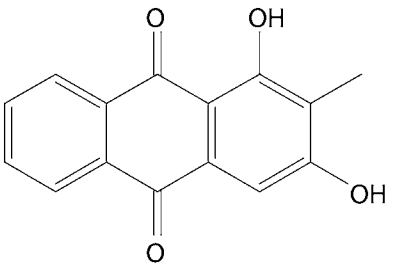
The contribution of Rubiaceae to Western pharmaceuticals and medicine is substantial because it is the source for *Uncaria gambier* (Hunt.) Roxb. (Catechu, *British Pharmaceutical Codex*, 1963), *Cephaëlis ipecacuanha* (Brot.) A. Rich. (*uragoga ipecacuanha*, Brazilian ipecacuanha), or *Cephaëlis acuminata* Karsten (*Cartagena ipecacuanha*) (Ipecacuanha, *British Pharmacopeia*, 1963), *Cinchona calisaya* (yellow

cinchona bark), *Cinchona ledgeriana* (ledger bark), *Cinchona officinalis* (pale cinchona bark, crown or Loxa bark), and *Cinchona succirubra* (red cinchona bark) containing quinine. Classical examples of Rubiaceae are *Coffea arabica* (Arabica coffee), *Coffea liberica*, and *Coffea canephora* (Robusta coffee).

In regard to the antineoplastic potentials of Rubiaceae, some evidence has already been presented that clearly demonstrates that anthraquinones inhibit the enzymatic activity of topoisomerase II. An example of antineoplastic anthraquinones that target topoisomerase II is mitoxantrone (Novatrone®), which is currently approved for clinical use in the United States (*16*). In the Pacific Rim, about 150 species of plants classified within the family Rubiaceae are medicinal, of which *Prismatomeris albidiflora*, *Knoxia valerianoides*, *Damnacanthus indicus*, and *Morinda umbellata* are known to produce anthraquinones. An interesting development from Rubiaceae would be to investigate its members for anthraquinones and assess them for topoisomerase inhibitors. The discovery of inhibitors of topoisomerase II of clinical antineoplastic value can be reasonably expected.

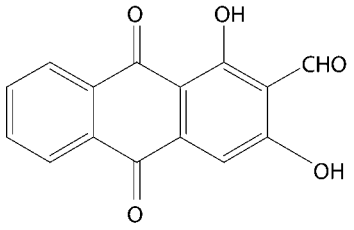



Mitoxantrone

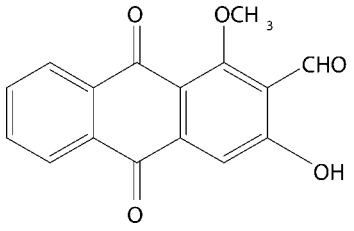



Anthraquinone

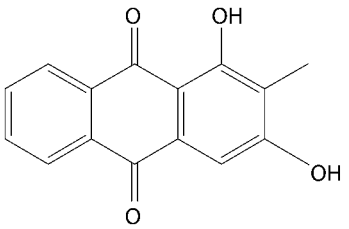
***Prismatomeris albiflora* Thaw, non King**, (*Prismatomeris tetrandra* [Roxb.] K. Sch, *Prismatomeris malayana* Ridl, *Coffea tetrandra* Roxb.), or *son kraal*, *duck kai dam* (Thai), is a treelet that grows to a height of 3 m in the rainforest, on rocky seashores, and in limestone rocks of Vietnam, Burma, Thailand, and Malaysia. The stems show internodes with a median longitudinal ridge ending between each pair of petioles. The leaves are simple,



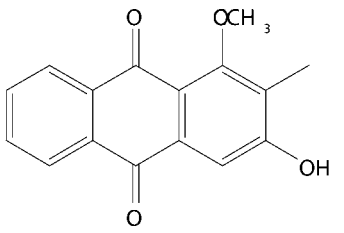

Nordamnacanthal




Damnacanthal




Rubiadin

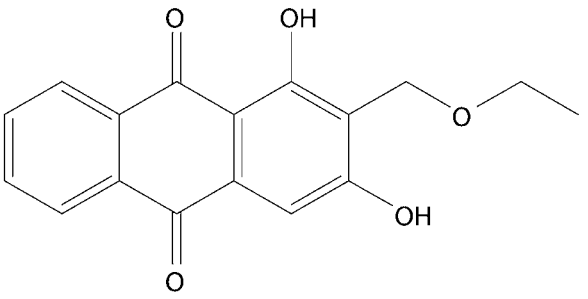



Rubiadin-1-methyl ether

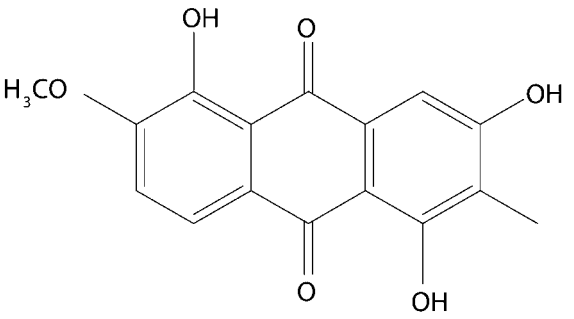
Fig. 85. Cytotoxic anthraquinones of *Prismatomeris fragran*.

chartaceous decussate, and stipulate. The stipules are interpetiolar, triangular, and bifid at the apex. The blade is ovate and elliptic and shows 6–10 pairs of secondary and tertiary nerves. The influorescences are terminal and axillary clusters. The flower pedicels are 6 mm–2.1 cm long. The calyx cup is fringed. The corolla is white, tubular, and four- to five-lobed. The fruits are globose and contain one to two grooved seeds.

In Malaysia, a paste of the leaves is used to heal wounds. In Cambodia, Laos, and Vietnam, a decoction of roots is drunk to treat bronchitis, and an infusion of wood is drunk to invigorate and to expel impurities. Malays apply a paste of leaves to wounds to promote healing. The pharmacological potential of *P. tetrandra* (Roxb.) K. Schk is unexplored. Note that the plant is known to produce anthraquinones such as rubiadin *(17)*. Kanokmedhakul et al. made the interesting observation that the roots and stems of *Prismatomeris fragrans* contains a series of anthraquinones, of which nordamnacanthal, damnacanthal, rubiadin, and rubiadin-1-methyl ether abrogated the survival of exhibited cytotoxicity to the NCI-H187 cell line cultured in vitro *(18)*. It would be interesting to learn about the topoisomerase activity of these anthraquinones (Fig. 85).




Ibericin




Knoxiadin

***Knoxia valerianoide* Thorel**, or knoxia root, Peking spurge root, or *Hung ya ta chi* (Chinese), is an herb that grows to a height of 60 cm in China, Cambodia, Laos, Vietnam, and North India. The roots are tuberous. The leaves are opposite and stipulate, and the blade is lanceolate and shows six to eight pairs of secondary nerves. The flowers are tubular, minute, and four-lobed (Fig. 86). In China, the plant is used to treat ailments related to excretion and to treat dropsy, but is not recommended during pregnancy. In Cambodia, Laos, and Vietnam, it is used to promote the fermentation of alcohol of rice.

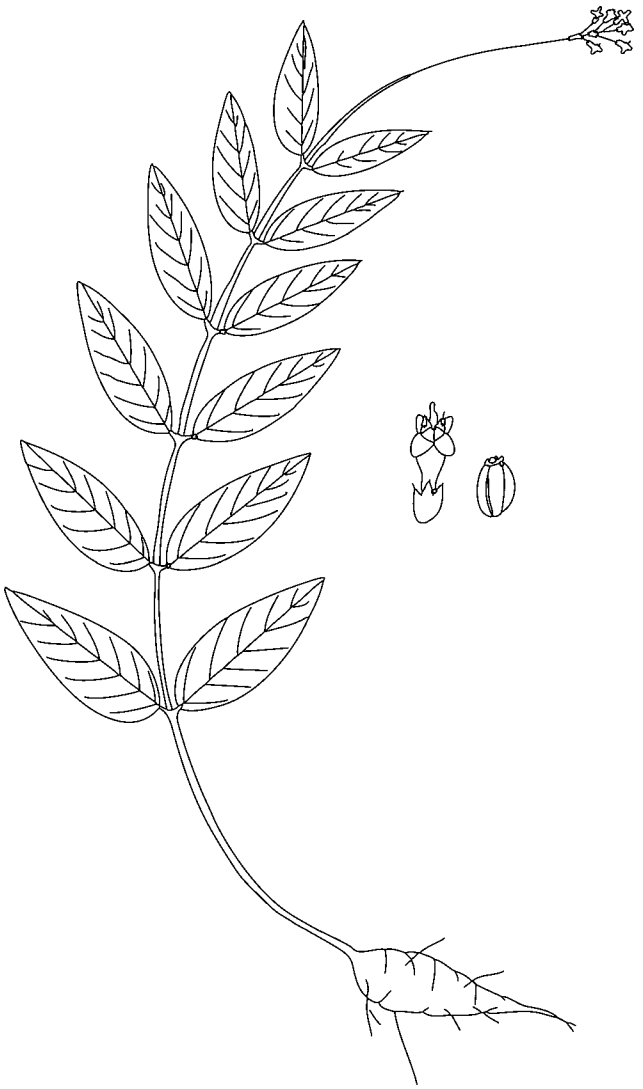


Fig. 86. *Knoxia valerianoide* Thorel.

The pharmacological property of *K. valerianoides* Thorel. is unexplored, but the plant is known to produce anthraquinones including 2-ethoxymethylknoxiavaledin, 2-formylknoxiavaledin, 2-hydroxymethylknoxiavaledin, knoxiadin, damnacanthal, nordamnacanthal;, ibericin, 3-methylalizarin, and damnacanthol (*19*).

***D. indicus* Gaertn.** is a little shrub that grows in a geographical zone spanning from the Himalayas, North India, China, Japan, and the Philippines. The stems are terete, minutely hairy, and develop slender, interpetiolar, stipular woody thorns. The leaves are opposite and simple. The blades are broadly lanceolate, thick, dark green and glossy above, and light green below. The base of the blade is cordate and the apex is apiculate.

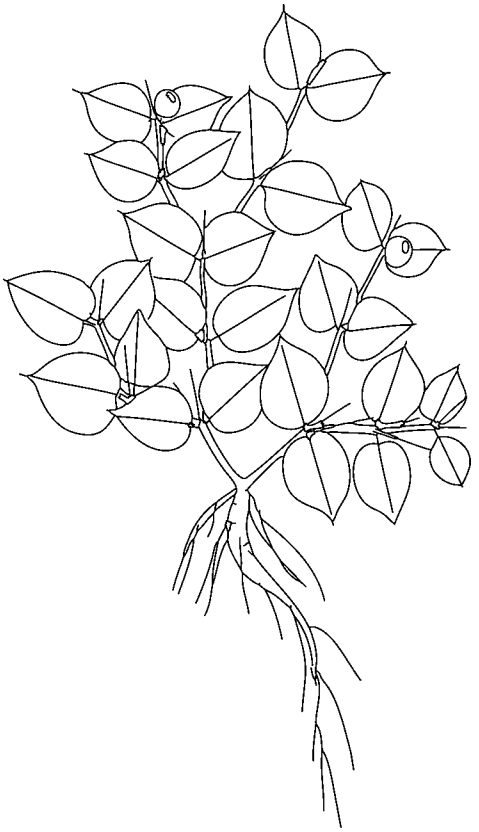


Fig. 87. *Damnacanthus indicus* Gaertn.

The flowers are white and in pairs. The fruits are red berries at axil of leaves (Fig. 87). In China, it is used to treat rheumatism, mitigate headache, and heal piles.

*D. indicus* Gaertn. is known to abound with anthraquinones, but its pharmacological potential remains unexplored to date *(20,21)*. Note that damnacanthal is a common component of the *Damnacanthus* species. Faltynek et al. made the interesting observation that damnacanthal inhibits the enzymatic activity of tyrosine kinase, which is involved in the propagation of metastases *(22)*. An interesting development from this observation would be to assess the topoisomerase inhibitory activity of the *Damnacanthus* species, an activity that could be associated with tyrosine kinase inhibition, hence enormous chemotherapeutic potentials.

***Neonauclea pallida* (Reinw. ex Havil.) Bakh f.** (*Nauclea pallida* Reinw. ex Havil, *Nauclea purpurascens* sensu K. & G., *Neonauclea calycina* sensu Corner.), or hooded bur-flower tree, *bengkal batu* (Malay, Indonesian), or *krathum khao* (Thai), is a bush that grows to 2 m tall in Burma, Thailand, the Andamans, Sumatra, Java, and Borneo. In Indonesia, the leaves are used externally to promote urination. The stem is glabrous. The leaves are simple, opposite, and stipulate. The blade is glabrous, chartaceous, 16 × 8 cm, with 8–12

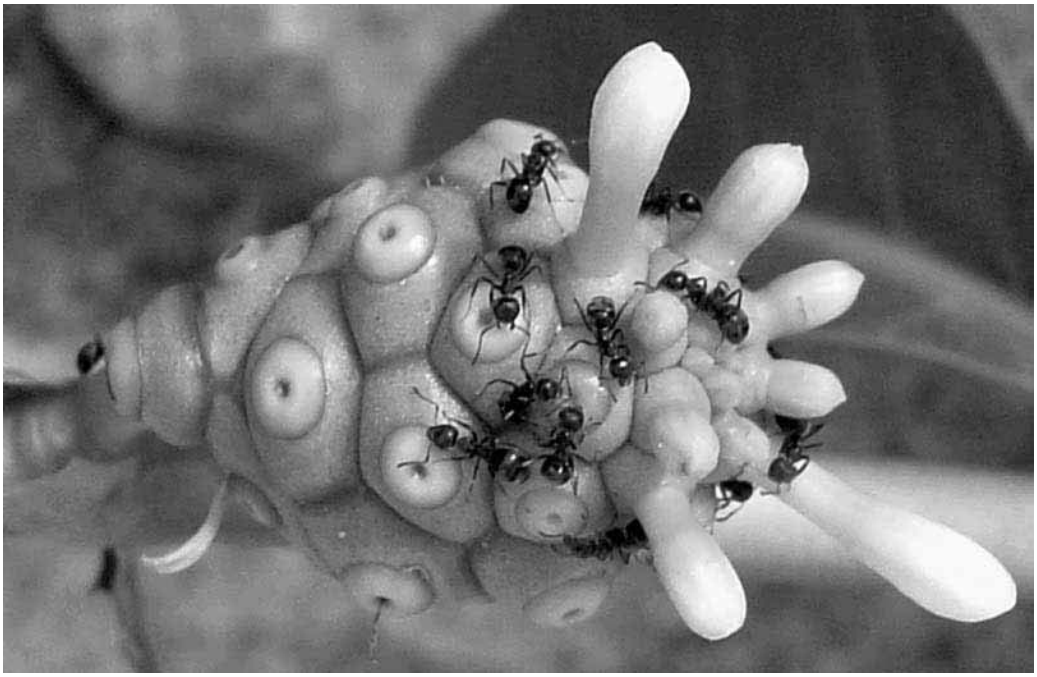


Fig. 88. Botanical hallmark of *Morinda* species: syncarps.

pairs of secondary nerves. Fruiting peduncle is axillary and terminal; the young flowering heads are enclosed in a pair of bracts. A wood extract of this plant afforded a series of anthraquinones including damnacanthal and morindone, which inhibited the enzymatic activity of topoisomerase II with $IC_{50}$ values of 20 and 21 μg/mL, respectively (*21*).

***Morinda officinalis* How**, or *pa chi t'ien*, *ba ji tian*, *pa kit tian* (Chinese), *hagekiten* (Japanese), or *p'agukch'on* (Korean), is a shrub native to China, mainly the Guangdong, Guangsi, and southern Fujian provinces. Note that *Morinda* fruits are easily recognized syncarps (Fig. 88). The drug consists of the roots and is sweet and acrid in taste, fawn outside and moniliform, and purplish yellow-white inside. In China, the roots are used to treat beriberi, quiet the visceral organs, regulate urination, treat rheumatic conditions, fight sterility, and increase mental power. The roots should not be taken by pregnant or lactating women. The root of M. *officinalis* How. abounds with series of anthraquinones including rubiadin, rubiadin-1-methyl ether, 1-hydroxy- anthraquinone, 1-hydroxy-2-methylanthraquinone, 1,6-dihydroxy-2,4-dimethoxyanthraquinone, 1,6-dihydroxy-2-methoxyanthraquinone, 1-hydroxy-2- methoxyanthraquinone physcion, 2-methyl-anthraquinone, and damnacanthal (*21*). Damnacanthal is widespread in *Morinda* species and known to inhibit the enzymatic activity of tyrosine kinase and topoisomerase (*22*,*23*). Hiwasa et al. noted that human fibroblast UVr-1 cells treated with damnacanthal prior to ultraviolet irradiation presented more DNA fragmentation (*24*). Using immunoblot analysis, they showed that pretreatment with damnacanthal followed by ultraviolet

irradiation increased the levels of phosphorylated extracellular signal-regulated kinases and stress-activated protein kinases, suggesting a stimulation effect of damnacanthal on ultraviolet-induced apoptosis. In regard to the stimulant property mentioned above, it is interesting to mention that the plant extract (25–50 mg/kg) showed antidepressant properties in rats similar to clinically antidepressant drug desipramine (5–10 mg/kg *[25]*). This activity is, to date, believed to be mediated by oligosaccharides. Li et al. *(26,27)* showed that oligosaccharides ($P_6$) protected PC12 cells from corticosterone-induced apoptosis in a concentration- and time-dependent manner. Can we reasonably expect oligosaccharides to cross the hemato-encephalic barrier and act in the brain? Probably not.

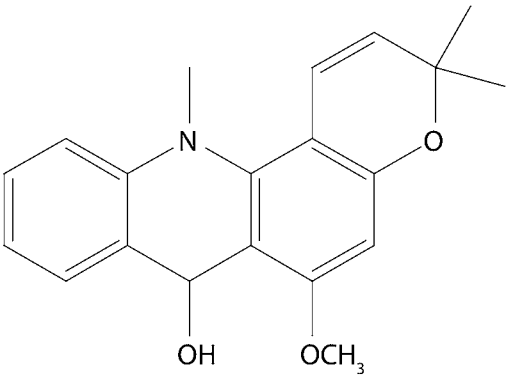
## Medicinal Rutaceae

The family Rutaceae consists of 150 genera and 1500 species of treelets known to accumulate essential oils (limonene), limonoids, flavonoids (hesperidin), coumarins, and several sorts of alkaloids including, notably, carbazole and acridone alkaloids. The cardinal features to note in field collection are lemon-like aroma of crushed leaves, a blade dotted with several translucent oil glands, white flowers with retrorse petals and conspicuous globose and light green stigma, and green lemon-like fruits. The fruits of several species in this family are edible: lemon (*Citrus limon* [L.] Burm. f.), sour orange (*Citrus aurantium* L.), sweet orange (*Citrus sinensis* [L.] Osbeck), and lime (*Citrus aurantifolia* [Chaistm.] Swingle).

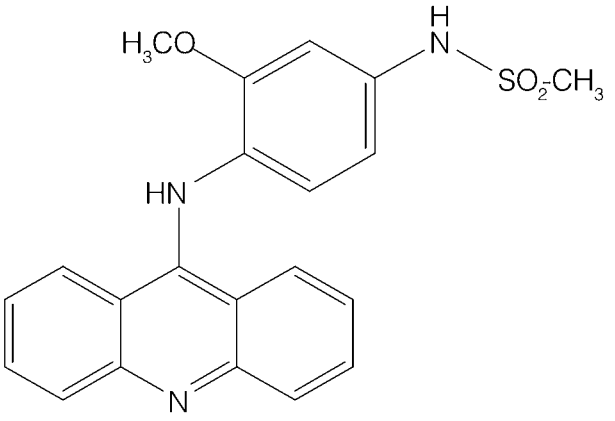
Western medicine has been using the essential oil of several species of Rutaceae as flavoring ingredient. The essential oil of *C. sinensis* (orange oil, *Oleum aurantii*, *British Pharmaceutical Codex*, 1963) has been used as a flavoring agent and in perfumery. Bergamot oil (*Oleum bergamottae*, *British Pharmaceutical Codex*, 1949) from *Citrus bergamia* has been used in perfumery in preparations for the hair (cologne spirit or *Spiritus coloniensis)*, lemon oil (*Oleum limonis*, *British Pharmaceutical Codex*, 1963) from *C. limon*, *Citrus limonia*, and *Citrus medica* is carminative and used as a flavoring agent, and the dry peel of *C. aurantium* (*Aurantii cortex siccatus*, *British Pharmacopoeia*, 1963) has been used as a flavoring agent and for its bitter and carminative properties. The oil of *Ruta graveolens* L. (common rue, herb of grace) has been has been used to stop spasms, promote menses, and produce skin irritation (rue, *British Pharmaceutical Codex*, 1934).

Examples of alkaloid of relative pharmaceutical usefulness so far characterized from Rutaceae are pilocarpine from *Pilocarpus jaborandi* Holmes. This imidazole alkaloid is occasionally used to treat glaucoma.

In regard to the antineoplastic properties of Rutaceae, this family has attracted a great deal of interest for its ability to elaborate series of cytotoxic benzo[c]phenanthridine and acridin alkaloids. Examples of acridin alkaloid are pyrano-acridone and acronycine characterized from *Acronychia baueri* Scott.




Acronycine




Amsacrine (Amsa P-D®)

Examples of acridin alkaloids used in therapeutics as antineoplastic agents is amsacrine (Amsa P-D®), which is used for the treatment of acute leukemia in adults and malignant lymphomas, refractory to conventional therapy. Amsacrin is an intercalating agent and topoisomerase II inhibitor.

One can reasonably envisage the family Rutaceae as a sockhouse of acridin-like alkaloids that await experimentation as inhibitors of topoisomerase and expect the discovery of antineoplastic agents of clinical value from this family. Looking for such agents, one might look into the medicinal Rutaceae of the Asia–Pacific region, which encompasses about 120 species of plants including *Zanthoxylum ailanthoides* Sieb. & Zucc, *Zanthoxylum bungei* Planch, *Zanthoxylum piperitum* (L.) DC, and *Zanthoxylum schinifolium* Sieb. & Zucc.

***Z. ailanthoides* Sieb. & Zucc.** (*Fagaras* [Sieb. & Zucc.] Engl.), or Japanese prickly ash; *karasuzanshou* (Japanese), *shih chu yii*, *yueh chiao*, or *la tzu* (Chinese), is deciduous small tree that grows to a height of 18 m in Japan, Korea, and China. The bark is grayish-brown

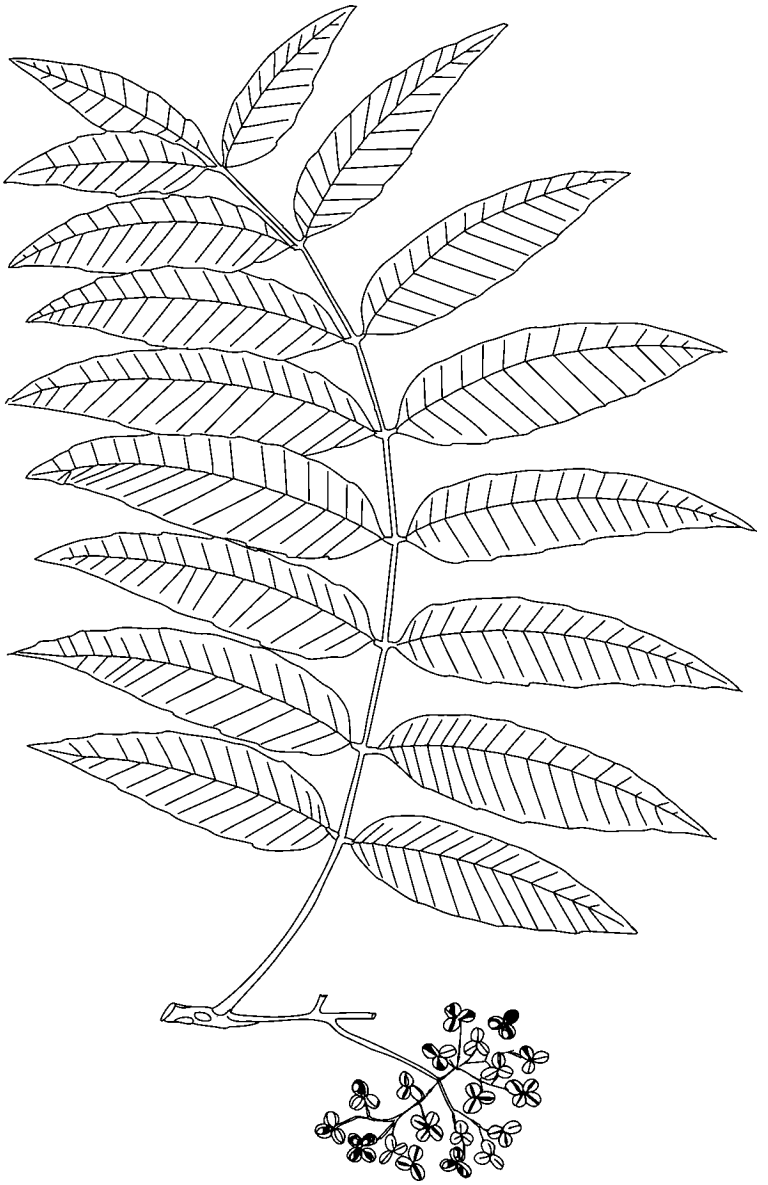
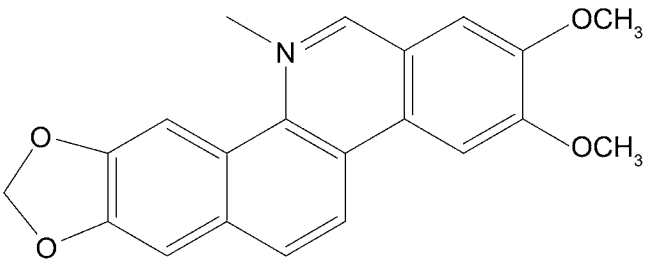


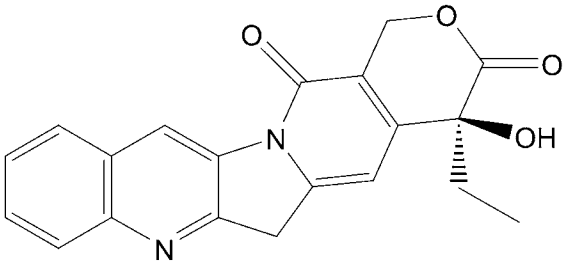
Fig. 89. *Zanthoxylum ailanthoides* Sieb. & Zucc.

and shows numerous small lenticels and is mottled with dark brown scars of thorns. The leaves are alternate, thick, and odd-pinnate. The folioles are linear-lanceolate, somewhat crenate, and show about 15 pairs of secondary nerves. The fruits are follicles, by three that open to show glossy black seeds of 5–8 mm in diameter (Fig. 89).
The follicles are green, pungent, and used as pepper. The fruits are eaten to promote digestion, as tonic, to counteract poisoning, treat sunstroke, diarrhoea, leucorrhea, and

dysentry. An infusion of leaves is drunk to treat chills and flux. The pharmacological property of this plant is as-yet unexplored. Note that the *Zanthoxylum* species are known to elaborate fagaronine and congener.




Nitidine




Camptothecin

Fagaronine and nitidine from *Zanthoxylum* species represent two of the more potent antitumor benzo[c]phenanthridines of Rutaceae. Both of these alkaloids have been shown to inhibit the enzymatic activity of topoisomerase in a way similar to camptothecin *(28,29)*. It would be interesting to learn whether further study on *Z. ailanthoides* Sieb. & Zucc. discloses any benzo[c]phenanthridine alkaloid of anti-neoplastic value.

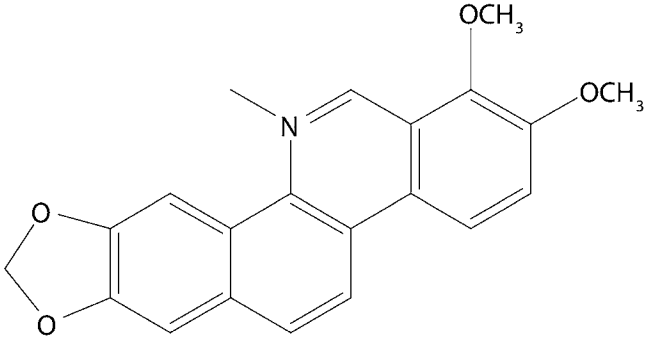
***Zanthoxylum bungei* Planch.** (*Zanthoxylum simulans* Hance.), or Szechuan pepper, Chinese pepper, Szechuan peppercorn, Sichuan pepper, Chinese prickly ash, *jiao mu ch'in chiao*, *ta chiao*, *hua chiao*, *chuan jiao* (Chinese), *sokusho* (japanese), or *ch'onch'o* (Korean), is a deciduous shrub growing to 6 m tall. The plant is native to China and Taiwan. The bark shows stout, woody, horizontal thorns. The leaves are compound-spiral and exstipulate. The blade comprises three pairs of folioles plus a terminal one. The lateral folioles are broadly elliptic, crenate, punctured with numerous oil translucent oil cells, and show four to five pairs of secondary nerves. The rachis is minutely winged. The fruits consist of 3–4-mm reddish-brown tuberculate follicles that are split open to show a black seed (Fig. 90).

The drug consists of the small, red tuberculate follicles enclosing black, round glossy seeds, which are aromatic, pungent, with a somewhat acrid aftertaste. It is a carminative and a stimulant, it promotes sweating, is an emmenagogue, astringent, and an

Fig. 90. *Zanthoxylum bungei* Planch.

anthelmintic, and is used as a condiment of high value for vital process. An infusion of carpels in vinegar is instilled in ears to remove insects or worms.

The plant is known to contain chelerythrine chloride, which inhibits the aggregation of rabbit platelet in vitro via inhibition on thromboxane formation and phosphoinositides breakdown (*30*). Chelerythrine, which occurs in members of the family Papaveraceae, has been reported to inhibit the enzymatic activity of protein kinase C and to exert cell-growth inhibitory effect via the induction of apoptosis in numerous cancer cell lines (*31,32*). What is the topoisomerase activity of chelerythrine?




Chelerythrine

***Zanthoxylum piperitum* (L.) DC.**, or Japanese peppercorn, Japanese pepper tree, Sichuan pepper, *hajikami*, *sanshou* (Japanese), *shu chiao*, *ch'uan chiao*, or *nan chiao*

(Chinese), is a spiny, deciduous, thorny shrub that grows to 3–4 m in height in China, Japan, and Korea. The leaves are compound, spiral, and exstipulate. The blade consists of six to seven pairs of folioles, which are thick and glossy, about 3–6 cm long, elliptic-ovate, resinous, fragrant, and crenate. The rachis is thorny. The fruits are 4 mm in diameter, on terminal panicles, bright red becoming purple when ripe, and split in two to free a single, black, glossy, seed that is pungent and tastes like lemon or ginger (Fig. 91). The drug consists of the dried follicles, which are used to stimulate digestion, malaria, dysentry, spermatorrhea, galactorrhea, excessive urination, and as a tonic. The carpels are used eternally to expel parasites. The seeds are eaten to promote urination, treat dropsy, invigorate kidneys and bladder, and treat asthma. The seeds are used in China and Japan as a substitute for pepper. The pharmacological potential of this plant is unexplored.

***Zanthoxylum schinifolium* Zieb. & Zucc.** (*Zanthoxylum schinnifolium*), or Korean pepper, *yai chiao* or *yeh chiah* (Chinese), is a tree that grows in China, Japan, and Korea. The trunk is armed with broad, fattened, woody, horizontally arranged thorns. The leaves are compound, spiral, and exstipulate. The blade shows three to five pairs of folioles. The folioles are lanceolate, asymmetrical, minutely serrate, and bright green. The flowers are minute and greenish-white in panicles. The fruits are pink and split to reveal a very aromatic gray and dull seed.

The follicles are used to treat asthma and cough and to mitigate painful swollen breasts. A paste of the leave is applied to contusion. Essential oil distilled from the follicles induced apoptotic death in HepG2 human hepatoma cells in a concentration- and time-related manner, and inhibited tumor development of mice inoculated with Huh-7 human hepatoma cells *(33)*.

## Medicinal Euphorbiaceae

The family Euphorbiaceae contains 300 genera and about 7500 species of trees, shrubs, herbs, and climbers, about 150 species of which are of medicinal value in the Asia–Pacific region. Most of these are used to relieve the bowels from costiveness, promote urination, soothe inflammation, and promote expectoration. A cardinal feature of Euphorbiaceae, and especially *Excoecaria*, *Aleurites*, *Croton*, *Euphorbia*, *Hippomane*, *Hura*, and *Jatropha* species, is their ability to elaborate a series of complex diterpenoid esters of the tigliane, ingenane, or daphnane type, which impart drastic cathartics and ulcerating and and strongly allergizing properties. An example of such Euphorbiaceae is *Excoecaria oppositifolia*, the latex of which is a common cause of temporary blindness and anaphylactic shock in the lumberjacks of the Asia–Pacific region.

In regard to the antineoplastic potential, most of the evidence that has emerged from the last 30 years supports the fact that Euphorbiaceae represent a vast reservoir of cytotoxic agents, and one may reasonably expect the isolation of original anticancer agents from this family if enough work is done. A remarkable advance in the study of anticancer principles from Euphorbiaceae has been provided by Wada et al. *(34)*.

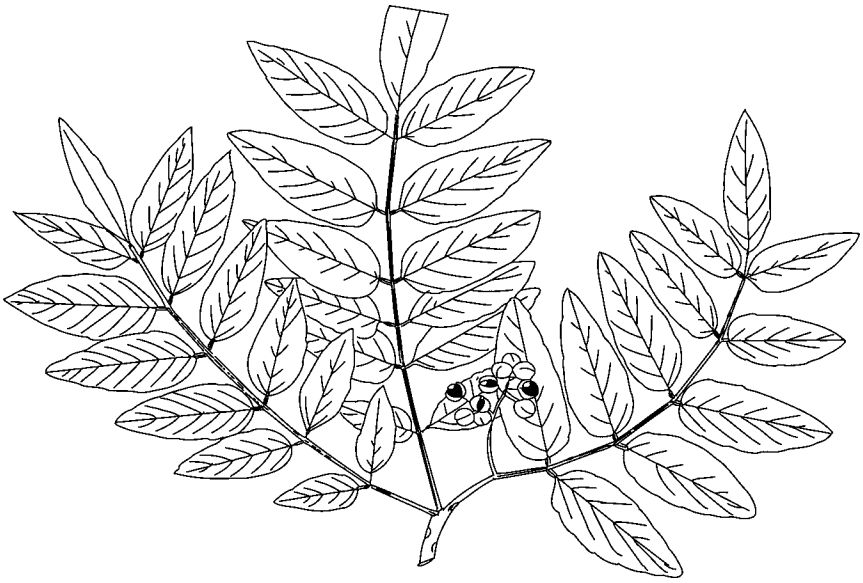
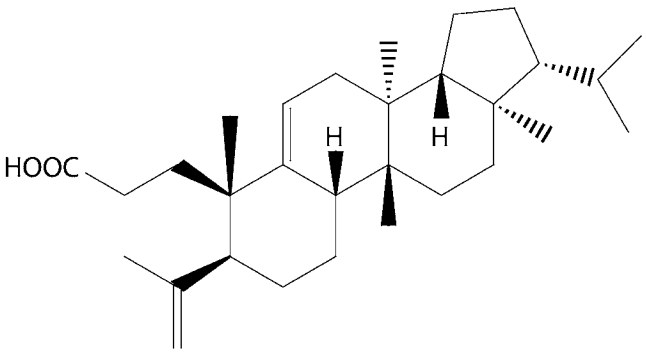


Fig. 91. *Zanthoxylum piperitum* (L.) DC.




3,4-Seco-8βH-ferna-4(23),9(11)-dien-3-oic acid

3,4-Seco-8βH-ferna-4(23),9(11)-dien-3-oic acid completely inhibited the enzymatic activity of topoisomerase II at concentrations up to 25 μM dose-dependently and more potently that etoposide.

***Alchornea villosa* (Benth.) Muell.-Arg.** is a small tree that grows to a height of 4 m in the primary rainforest of Malaysia. The stems are hairy when young and smooth. The leaves are simple, spiral, and stipulate; the stipules are linear and 5–7 mm long. The blade is hairy, lanceolate, membranaceous, 18.5 × 11.3 cm– 5 × 1.6 cm, and serrate. The fruits are capsular, bilobed, and show three stigmas at the apex that are 1.2–1.5 cm long (Fig. 92). In Malaysia, the plant is used as an antidote, and the leaves are used to calm itchiness of the skin. Indonesians have been known to drink the sap squeezed

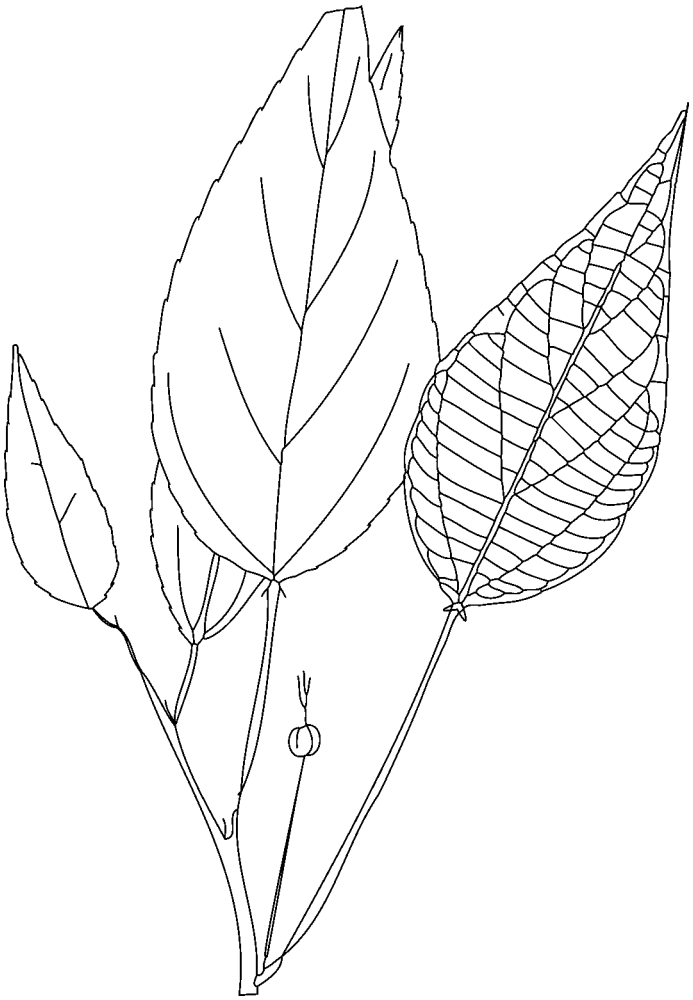
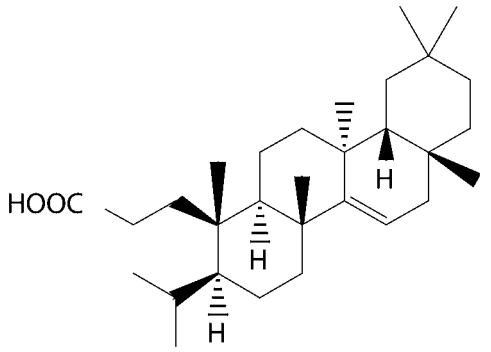


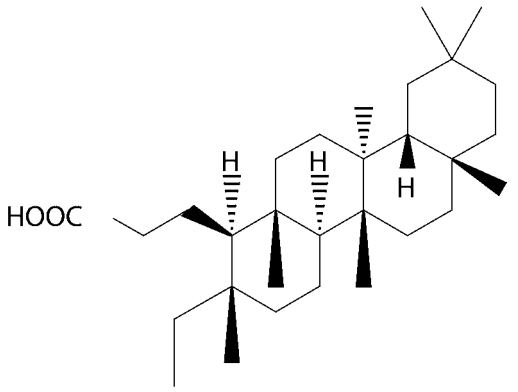
Fig. 92. *Alchornea villosa* (Benth.) Muell.-Arg. From KLU Herbarium 33970. Flora of Johore, West Malaysia. Comm. Ex. Herb. Hort. Bot. SING. Field collector & botanical identification: J.F. Maxwell. 4/10/1982. Geographical localization: Kota Tinggi Waterfalls.

from the young leaves to combat fever, and to apply a paste of leaves on the head to mitigate headaches and vertigo. The pharmacological and especially antineoplastic properties of *Alchornea villosa* (Benth.) Muell.-Arg. are, to date, unexplored. Note that seco-3,4 triterpenoids from the leaves of *Alchornea latifolia* are cytotoxic and inhibit topoisomerase II.

Seco-3,4-taraxerone and seco-3,4-friedelin 2 abrogated the survival of human hepatocellular carcinoma (Hep-G2) and human epidermoid carcinoma (A-431) cell lines cultured in vitro with $IC_{50}$ values of 11.7 and 38.2 mM, and inhibited the enzymatic activity of topoisomerase at dose of 7 μM (*35*).




1 seco-3,4-Taraxerone




seco-3,4-Friedelin

***Alchornea rugosa* (Lour.) Muell. Arg** (*Cladodes rugosa* Lour., *Alchornea hainanensis* Pax & K. Hoffm, *Alchornea javensis* [Endl. Ex Hassk.] M.A.) is a bush that grows up to 1.5 m tall in lowland rainforests of up to 300 m a geographical area spanning from South China, Indonesia, the Philippines, and Thailand. The leaves are simple and spiral. The blade is elliptic-lanceolate with a pair of stipels at base, hairy to scurfy below, and the margin is serrate. The fruits are up to 10 mm long. In Cambodia, Laos, and Vietnam, the seeds are eaten to relieve the bowels from costiveness. Malays use a decoction of roots and leaves to reduce fever and treat ague. The pharmacological potential of this plant is unexplored. It would be interesting to learn if further study of this plant discloses any triterpenoids of chemotherapeutic value.

***Phyllanthus acidus* (L.) Skeels** (*Cicca acida* [L.] Merr, *Cicca disticha* L, *Phyllanthus distichus* Muell.-Arg.), or Malay gooseberry, wild plum, *mayom* (Thai), *cerme* (Indonesian), *cermai* (Malay), *thinbozihpyoo* (Burma), *kântûet* (Cambodia), *nhôm baanz* (Laos), and *chùm ruôt* (Vietnam), is a small tree that grows to a height of 9 m tall. The plant is thought to have originated in Madagascar and is now widespread in tropical Asia. The leaves are compound and consist of about 12 pairs of folioles

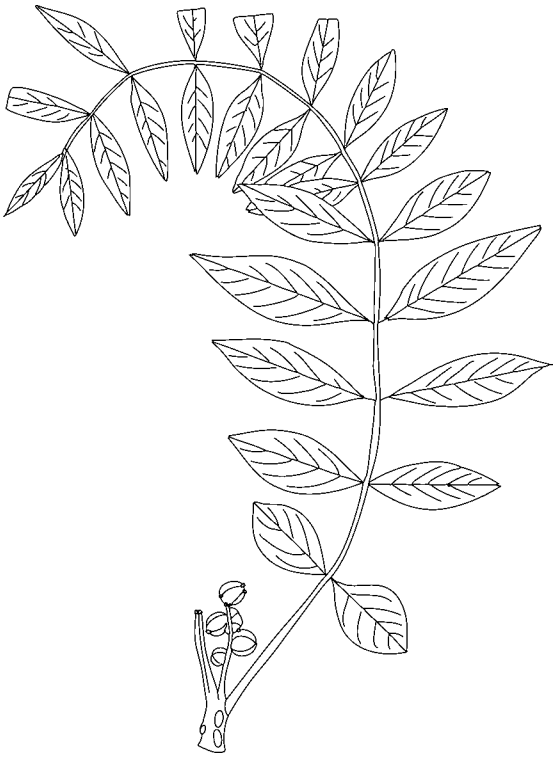
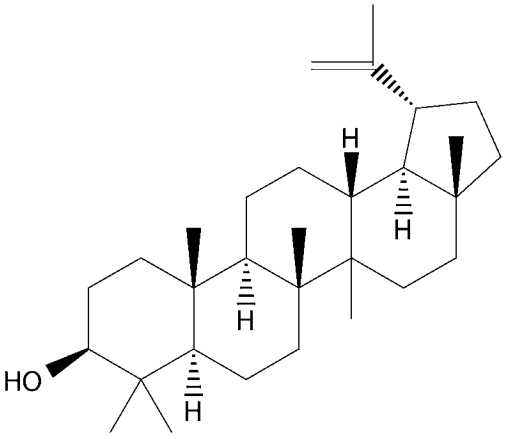


Fig. 93. *Phyllanthus acidus.* Forest Department herbarium, Brunei. Brun 5171. Geographical localization: kedayan, Cheramai. Jalan Muara, South Lumut. In old, secondary forest. 2/3/1959.

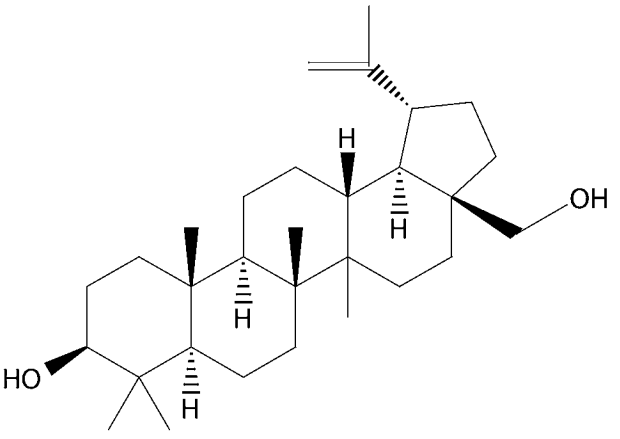
that are glaucous below and linear-lanceolate. The flowers are small, pinkish, and arranged in panicles up to 12 cm long. The fruits are cream colored, globose, six- to eight-lobed drupes of up to 2.5 cm in diameter that are palatable, apple-like in taste, sour, and containing four to six seeds (Fig. 93).

Burmese eat the fruits to promote appetite, and swallow the sap to induce vomiting and relieve the bowels from costiveness. In the Philippines, the leaves are used externally to calm itchiness, and a decoction of the bark is drunk to treat lung diseases. In Indonesia, the leaves are used as counter-irritant in sciatica and lumbago. In Malaysia, the vapors emitted when boiling the roots in water are inhaled to treat cough and headache.

The plant is known to produce norbisabolane diterpenes, including phyllanthusols A and B, which are both cytotoxic *(36)*. From the bark, pentacyclic triterpenoids, phyllanthol, and olean-12en-3β-ol (β-amyrin) have been isolated *(37)*. Note lupane- and oleanane-type triterpenoids isolated from the bark of *Phyllanthus flexuosus*, such as olean-12-en-3 β,15 α-diol, olean-12-en-3 β,15 α,24-triol, lupeol, and betulin inhibited the enzymatic activity of topoisomerase II activity with $IC_{50}$ values in the range of 10 to 39 μM *(38)*.




Lupeol




Betulin

Although the precise molecular mechanism of plant triterpenoids on topoisomerase II remains elusive, accumulated experimental evidence indicates that plant triterpenoids represent a vast potential source of antineoplastic agents. An interesting development from these results would be a massive survey of plant triterpenoids as source of topoisomerase inhibitors. Also, one might have noticed that plant triterpenoids have an affinity to phospholipase. Martelli et al. observed that during apoptosis, a phospholipase D-mediated signaling pathway operating at the nuclear level is elicited and may represent an attractive therapeutic target for the modulation of apoptotic events in human disease (39).

***Macaranga triloba* (Reinw.) Muell.-Arg.**, or *mahong merah* (Malay), is a treelet that grows wild to a height of 6 m in Malaysia, Burma, Thailand in secondary forests, often gregarious, gaps, and river valleys in primary forests. The stems are hollow with ants ribbed and constricted. The leaves are simple, spiral, and stipulate. The stipules are leathery and persistent. The blades are lobed, membranaceous, with scattered yellow granular glands and conspicuous nerves below (Fig. 94).

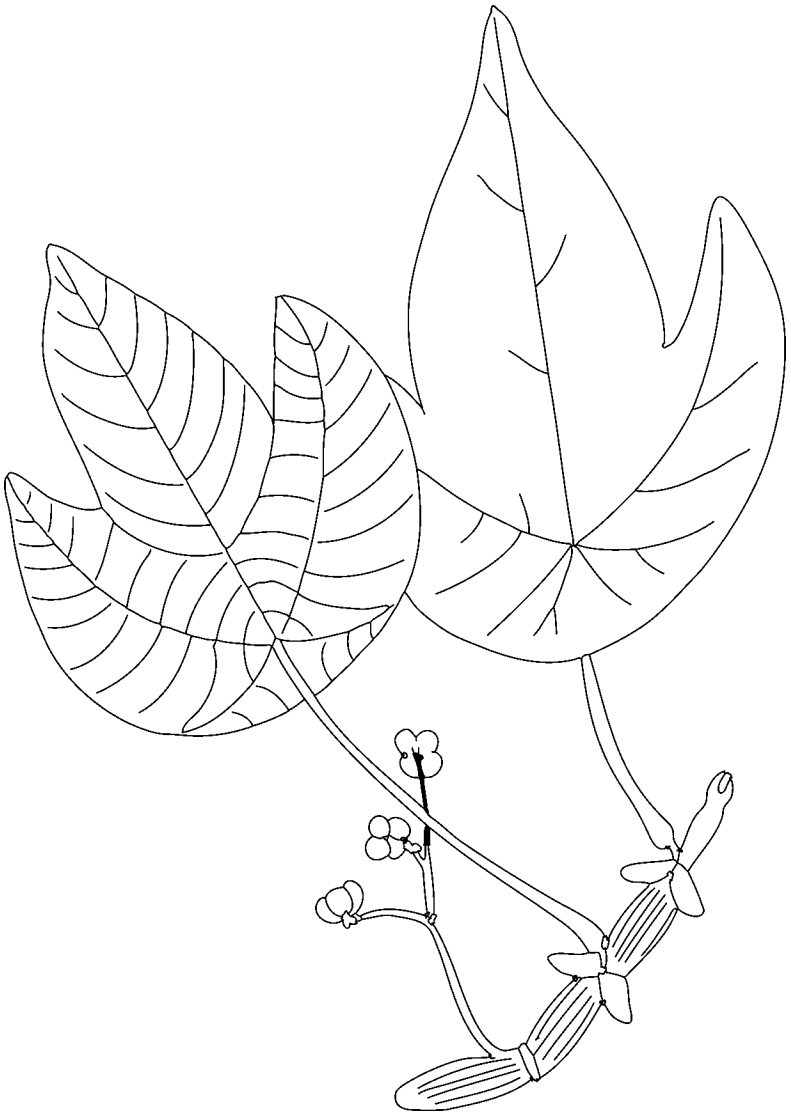
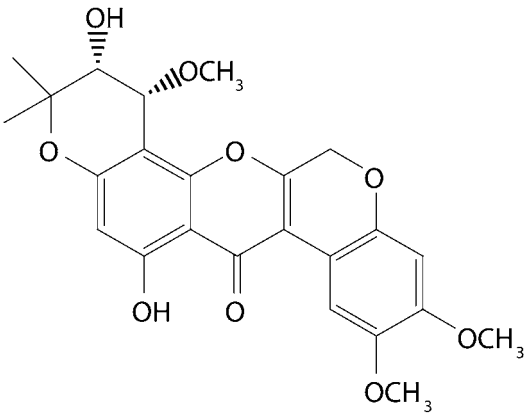


Fig. 94. *Macaranga triloba.* Flora of Malaya. FRI main road side, Kepong. Field collector: Matr. Asri.

In Malaysia, the leaves are used externally to heal boils. In Indonesia, a decoction of fruits and leaves is drunk to mitigate stomachache. The fruits are poisonous.
Activity-guided fractionation of the leaves of *Macaranga triloba* (Reinw.) Muell.-Arg. using an in vitro bioassay based on the inhibition of cyclooxygenase-2, resulted in the isolation of flavonoids, triterpenes, and of 4,5-dihydro-5′α-hydroxy-4′α-methoxy-6α,12α-dehydro-α-toxicarol *(40)*. The inhibition of cyclooxygenase 2 as an important mechanism for cancer chemoprevention has been supported by epidemiological and experimental evidence notably reported by Dannenberg et al. *(41)*.




4,5-dihydro-5'α-hydroxy-4' α -methoxy-6 α,
12 α -dehydro- α -toxicarol

It would be interesting to learn whether 4,5-dihydro-5′α-hydroxy-4′α-methoxy-6α,12α-dehydro-α-toxicarol interferes with the enzymatic activity of topoisomerase. Note that taxol, a successful chemotherapeutic agent that hampers the normal microtubule machinery, has been suggested to involve additional cytotoxic pathways including p53-independent apoptosis of tumor cells and inhibition of topoisomerase II (*42*).

## Medicinal Hamamelidaceae

The family Hamamelidaceae consists of 26 genera and about 100 species of shrubs or tress known to contain tannins and iridoids. The leaves are alternate, simple, and often palmately lobed. The flowers are small and appear in spikes. The fruits are woody, capsular, and scepticidal. In Western medicine, the dried leaves of *Hamamelis virginiana* (hamamelis, *British Pharmaceutical Codex,* 1963), yielding not less that 20% of alcohol (45%)-soluble extractive, have been used as astringents for the treatment of hemorrhoids. Hamamelis water (*British Pharmaceutical Codex*, 1969) made from the stems has been used as a cooling application to sprains and bruises and as a styptic remedy. It is also used in cosmetics and as active ingredient of eye lotions.

In the Asia–Pacific region, *Altingia excelsa* Noronha (*Liquidambar altingiana* Bl.), *Altingia gracilipes* Hemsl. (*Amyris ambrosiaca* L.f.), *Liquidambar orientalis* Mill, and *Liquidambar formosana* Hance are of medicinal value. The purified basalm obtained from the trunk of *Liquidambar orientalis* Mill. or prepared storax (*British Pharmaceutical* Codex, 1969) has been used similarly as Peru basalm in the form of an ointment to treat scabies and other skin diseases, and as an ingredient of Compound Benzoin Tincture.

***Altingia excelsa* Noronha** (*Liquidambar altingiana* Bl.), or *xi qing pi* (Chinese), is a tree that grows to a height of 20 m tall in a geographical area ranging from India, Bhutan, India, Indonesia, Malaysia, Burma, and Indonesia. Leaves are simple, alternate, and stipulate; the stipules are 2–6 mm long. The petiole is slender and sparsely pubescent. The blades are aromatic with a turpentine-like aroma, ovate, 8–14 cm × 3.8–7 cm, crenate,

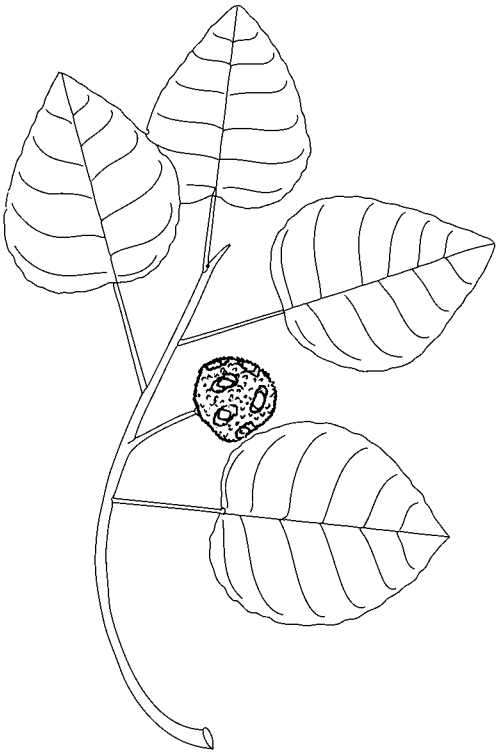


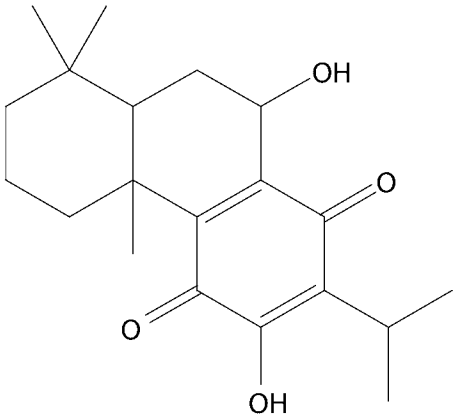
Fig. 95. *Altingia excelsa*. Plants of Indonesia. Herbarium Bogoriense—Harvard University Herbaria. Geographical localization: Bali, Timur, Tabanah 2 km west of Candi Kuning, in natural area of Kebun Raya, behind introduced Altingia forest. Alt: 1400 feet. 8° 18′S – 115° 9′E. Field collector: MacDonalds, 1961.

membranaceous, and show six to eight pairs of secondary nerves. The Infructescences are conical and 2 cm wide; and the fruits are capsules (Fig. 95).

In China, the plant affords a tonic remedy particularly recommended for chest complaints. Indonesians use the leaves medicinally because they are strongly aromatic. The pharmacological potentials of this plant are, to date, unknown. Note that 6β-hydroxy-3-oxo-lup-20(29)-en-28-oic acid and 3,11-dioxoolean-12-en-28-oic acid from the stem bark of *Liquidambar styraciflua*, as well as 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid, inhibited the growth of several cancer cell lines (*43*). What is the activity of 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid against topoisomerase?

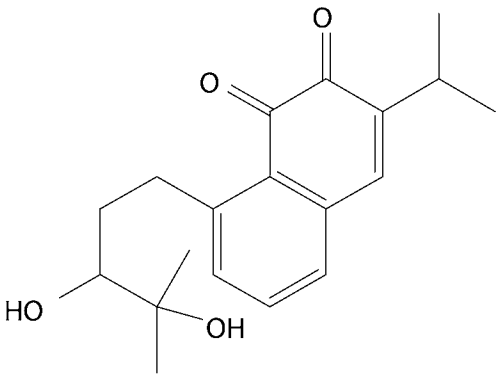
## Medicinal Lamiaceae

There is an expanding body of evidence to suggest that diterpene quinone of *Salvia* species might represent a pool of potential inhibitors of topoisomerase. Diterpenoid quinones have been characterized from *Salvia officinalis* (sage, *British Pharmaceutical Codex*, 1934), *Salvia texana*, *Salvia regal*, *Salvia moorciuftiana*, and *Salvia lanata* (*44–47*).




Horminone

Royleanone, horminone, and acetyl horminone isolated from the roots of *Salvia officinalis* L. abrogated the survival of colon carcinoma cell Caco-2 and human hepatoma cell HepG2, cultured in vitro with induction of DNA breaks *(48)*.

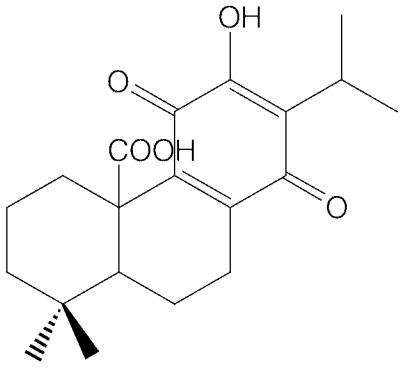



Salvicine

An other example of *Salvia quinone* is salvicine, a structurally modified diterpenoid quinone derived from *Salvia prionitis*, which is cytotoxic against multidrug-resistant cancer cell lines of topoisomerase II inhibition by trapping the DNA–topoisomerase II complex *(49)*.

***Salvia plebeia* R.Br.** (*Salvia plebia*, *Lumnitzeria fastigiata* [Roth] Spreng, *Ocimum fastigiatum* Roth, *Ocimum virgatum* Thunb., *Salvia brachiata* Roxb., *Salvia minutiflora* Bunge, *Salvia plebeia* var. *latifolia* E. Peter), or *ching chich*, *li zhi cao* (Chinese), is an annual or biennial herb that grows in a geographical area spanning from China, Korea, Afghanistan, India, Indonesia, Japan, Korea, Malaysia, Burma, Mongolia, Thailand, Vietnam, and Australia. The stems are erect, up to 90 cm, and stout. The blade is elliptic-ovate, minutely hispid, and serrate. The influorescences are pilose. The flowers are reddish-purplish to blue, and the nutlets are ovoid and minute (Fig. 96).

Fig. 96. *Salvia plebeia* R.Br.




Royleanolic acid

In China, the plant is used to counteract skin putrefaction, heal boils, treat catarrh, dispel humors, and stop dysentry. In Korea, the plant is used to treat anuria and expel intestinal worms. In Cambodia, Laos, and Vietnam, the plant is used to treat colic, cholera, and dysentery.

Fig. 97. *Salvia japonica* Thunb.

The plant is known to elaborate a diterpene quinone known as royleanonic acid, which is an antioxidant (50). What is the activity of royleanonic acid against topoisomerase?

***Salvia japonica* Thunb.**, or *shu wei ts'ao*, *wu ts'ao*, *shui-ch'ing*, *shu wei cao* (Chinese), is an annual herb that grows in marshes in China and Taiwan. The stems are erect, up to 60 cm, villous, and quadrangular. The leaves are pinnate. The petiole is 7–10 cm; the leaf blade is 6–13.5 × 5–9.5 cm; the upper stem leaves are one-pinnate. The flowers are packed in bunches on terminal racemes or panicles. The corolla is reddish to purple and 1.2 cm long. The nutlets are brown and minute (Fig. 97). The plant is used for black dye in China. The flowers and leaves are used to treat fluxes, colorless and red discharges, goiter and scrofula, ague, and dropsy. The rhizome is used for anemia, uterine hemorrhages, irregular menses, abdominal pains, inflammation, and as an antiseptic.

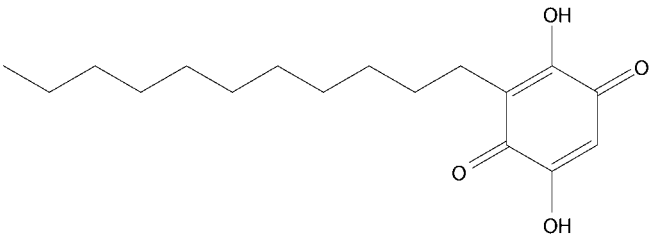
***Salvia miltiorrhiza* Bunge**, or red sage, *tan shen*, or *dan shen* (Chinese), is a common perennial herb of China, especially in hillsides, streamsides, and forests from 100 to 1300 m in Shensi, Shansi, Shantung, in the Peking mountains, and Japan. The leaves are hirsute. The root is red outside and purplish inside when fresh. The stems are erect, up to 80 cm tall, hirsute, and many-branched. Leaves are simple to imparipinnate and densely hirsute. The blade is circular to broadly lanceolate and crenate.

Fig. 98. *Salvia miltiorrhiza* Bunge.

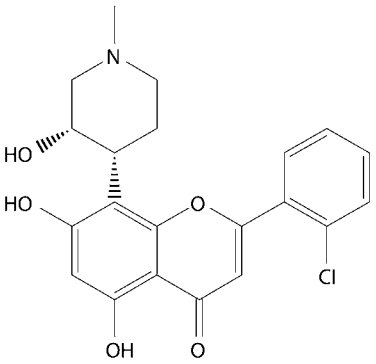
The influorescences are terminal or axillary. The flowers are tubular, bilabiate, purple-blue, violet, or white, and 2–2.7 cm and pilose. The nutlets are ellipsoid and 3.2 × 1.5 mm (Fig. 98).

The drug consists of dried short pieces of roots, which are brick red-colored, with a sweet taste resembling to liquorice. This herb is regarded as one of the five astral remedies by Chinese practitioners who recommend it for the cardiovascular system and blood-related ailments, such as hemorrhages and menstruation, and to promote healing of wounds.

Extract of the plant inhibited the proliferation of $HepG_2$ cells cultured in vitro and caused apoptotosis *(51)*. Chor et al. investigated the effects of a number of Chinese medicinal herbs on cellular proliferation and apoptosis of a rat hepatic stellate cell line, HSC-T6, and found that *Salvia miltiorrhiza* has antiproliferative and pro-apoptotic activities *(52)*. The active principle involved here might be a diterpene of the tanshinone type because tanshinone IIA, from *Salvia miltiorrhiza* Bunge, is cytotoxic against various human carcinoma cell lines cultured in vitro accompanied by an increase in intracellular calcium. This triggers the release of cytochrome c, thus causing a loss of the mitochondrial membrane potential, which results in the subsequent activation of caspases, hence apoptosis *(53)*.

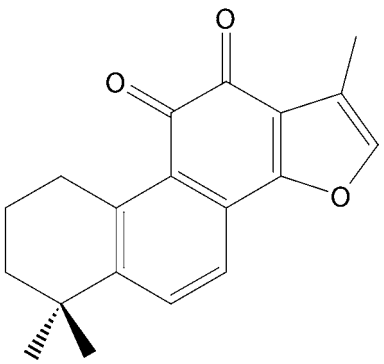



Embelin



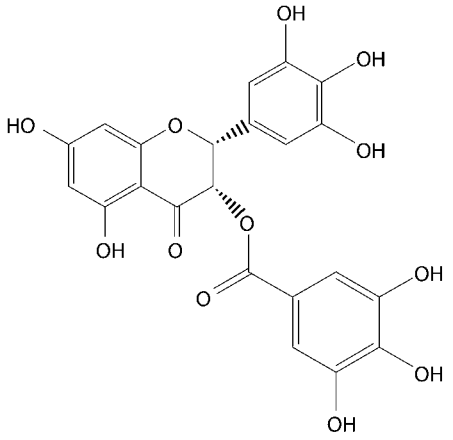

Flavopiridol

Fig. 99. Examples of natural products that interfere with apoptosis.

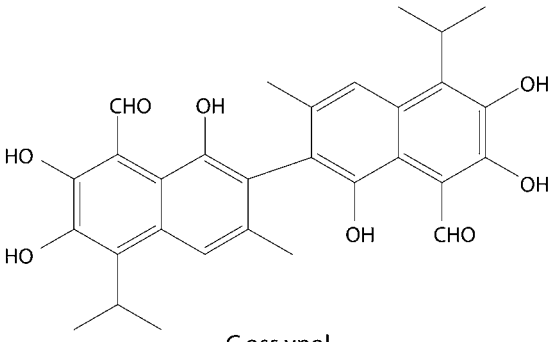



Tanshinone II

Tanshinones isolated from the dried root of *Salvia miltiorrhiza* Bunge abrogated the survival of P388 lymphocytic leukemia cells cultured in vitro. Tanshinone I and tanshinone IIA showed 86.76 and 56.05% cell inhibition, respectively, at a dose of 25 μg/mL) (*54*). Dihydrotanshinone I and cryptotanshinone were relatively cytotoxic (*55*). Dihydrotanshinone I induces cell growth arrest during the S phase and, subsequently, apoptosis.

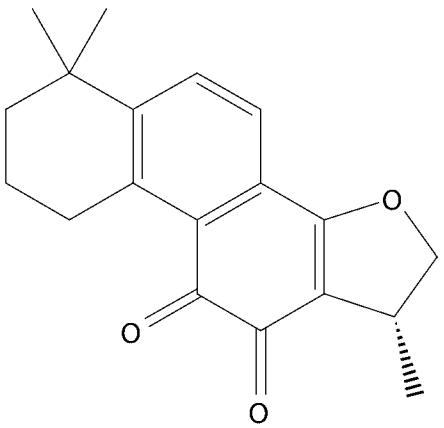



Epigallocatechin gallate




Gossypol

Fig. 100. (Continuation): Examples of natural products that interfere with apoptosis.




Cryptotanshinone

The evidence that emerged from the pharmacological study of *Salvia miltiorrhiza* Bunge lends strong support to the fact that tanshinone and congeners induce apoptosis. However, if the apoptotic potential of tanshinones is established, much less work has been done with the anti-topoisomerase activity of this planar compound. Further work on this topic should be encouraged, and one can reasonably expect interesting results not only in terms of antineoplastic agents but also in regard to a better understanding of the actual relationship between topoisomerase inhibition and apoptosis. What is the relationship between topoisomerase inhibitors and apoptosis induction?

## APOPTOSIS

In some cells, drug treatment and other stimuli can trigger a series of complex cytoplasmic biochemical reactions that appear to constitute a cellular suicide program, culminating in the degradation and compaction of chromatin. This programmed mode of cell death is known as apoptosis, and in normal cellular conditions, it plays a considerable role in the early development of homeostasis of adult tissues. Apoptosis or literally "programmed cell death," is so far known to be triggered by three major stimuli: cell surface receptors such as FAS, mitochondrial response to stress, and cytotoxic T-cells.

The Fas receptor (CD95) mediates apoptotic signaling by Fas ligand expressed on the surface of other cells. Binding of FAS to Fas ligand activates apoptotic signaling through activation of a series of cytoplasmic enzymes called caspases. Caspases are cysteine proteases that convey the apoptotic signal by cleaving and activating other caspases, which then degrade DNA. Caspase-8 is the initial caspase involved in response to receptors with a death domain such as FAS. The mitochondrial stress pathway begins with the release of cytochrome *c* from mitochondria, which then interacts with Apaf-1, causing self-cleavage and activation of caspase-9, which activates caspase-3, -6 and -7, which act themselves to cleave cellular targets. One mechanism used by cytotoxic T-cells to kill tumor cells and virus-infected cells is the release of perforin and granzyme proteins. Granzyme B and perforin proteins released by cytotoxic T-cells induce apoptosis in targeted cells, forming transmembrane pores, and trigge apoptosis through cleavage of caspases. For the past two decades, there has been a considerable amount of research of agents that interfere with apoptosis, and natural products, most of which are elaborated in Asian plant species, are being scheduled for clinical trials, such as theaflavins from tea, flavonoid derivative flavopiridol, epigallocatechin gallate, gossypol, and embelin (Figs. 99, 100) (*56–60*).

Embelin, which is common in the family Myrsinaceae in plants such as *Embelia ribes* Burm f., is of particular interest because it binds to the BIR3 domain of X-linked mammalian inhibitor of apoptosis protein (XIAP) and competes with caspase-9. Embelin inhibits cell growth, induces apoptosis, and activates caspase-9 in prostate cancer cells with high levels of XIAP, but has a minimal effect on normal prostate epithelial and fibroblast cells with low levels of XIAP and represents a promising lead compound for designing an entirely new class of anticancer agents that target the BIR3 domain of XIAP.


Fig. 101. Typical flower of *Goniothalamus* (*Goniothalamus scortechinii* King). The figure shows the veined petals and sepals.

One might have noticed that most of these natural products are of a phenolic nature and we can reasonably expect, in light of the complexity of apoptosis and the broad spectrum of cytotoxic agents elaborated from flowering plants, the discovery of lead apoptotic–antineoplastic agents in the relatively near future. When looking for such agents, one could investigate the medicinal flora of the Asia–Pacific region.

## Medicinal Annonaceae

The evidence currently available strongly suggests that members of the family Annonaceae hold some potential as sources of proapoptotic agents. Of particular interest in this regard is the genus *Goniothalamus*. The main macroscopically botanical characteristics to look for in field collection of *Goniothalamus* are treelets or shrubs with smooth, thin, and fibrous bark; aromatic wood; simple, alternate, and exstipulate leaves, with long glossy blades, and without obvious secondary nerves; a triangular thalamus or receptacle; and a perianth, which consists of a calyx, and two series of three greenish petals that are veined and somewhat woody (Fig. 101). Lens examination of the corolla shows that the inner whorl of the petal is smaller than the outer one and is fused in a vault above the androecium. The fruits are one- to two-seeded ripe carpels that are olive green and smooth.

Within the Annonaceae, *Goniothalamus* species are thought to have evolved from the tribe Uvariae, from which they have possibly inherited the ability to elaborate styryl-lactones and acetogenins, the former possibly being present in several other genera in the tribe Annoneae and known to occur in other Magnoliidae, including the Laurales and Piperales.

***Goniothalamus* species.** Six *Goniothalamus* species are used in the traditional medicinal Asian system, and most of these have been used in Malaysia in connection with

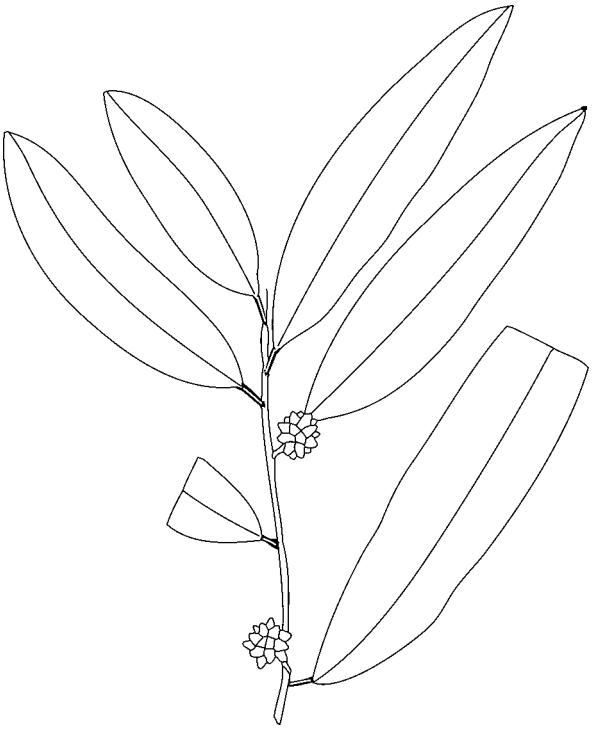


Fig. 102. *Goniothalamus macrophyllus* (Bl.) Hook. f. & Thoms.

abortion and childbirth since very early times. The leaves of *Goniothalamus macrophyllus* Hook. f. & Thoms. are used to abrogate fever, and a decoction of the roots is given as a postpartum remedy and to cause abortion. The roots of *Goniothalamus giganteus* Hook. f. & Thoms. (Fig. 102) are used to abort and treat colds, and the heated leaves are applied to swellings. A decoction of *Goniothalamus scortechinii* King is given as a postpartum protective remedy. The roots of *Goniothalamus tapis* Miq. (Fig. 103) are used as an abortifacient during early months of pregnancy. In Indonesia, an infusion of the roots is used to treat typhoid fever. In Taiwan, the seeds of *Goniothalamus amuyon* Merr. are used to treat scabies. In the Philippines, the seeds are used to treat rheumatism and tympanites, and the fruit is stomachic. None of the uses mentioned here has been substantiated yet via pharmacological experimentation; however, these species are well-known for their phytochemical constituents, as all six have been phytochemically investigated. Most phytochemical reports found so far on *Goniothalamus* species deal with the chemical constituents of *Goniothalamus giganteus* Hook. f. & Thoms. Phytochemical studies on *Goniothalamus* species have resulted, so far, in the isolation of two major classes of lipophilic secondary metabolites: acetogenins and styryl-lactones, both possessing complex stereochemistry and existing in different stereoisomeric forms with varying levels of toxicity toward several human tumor cell lines.

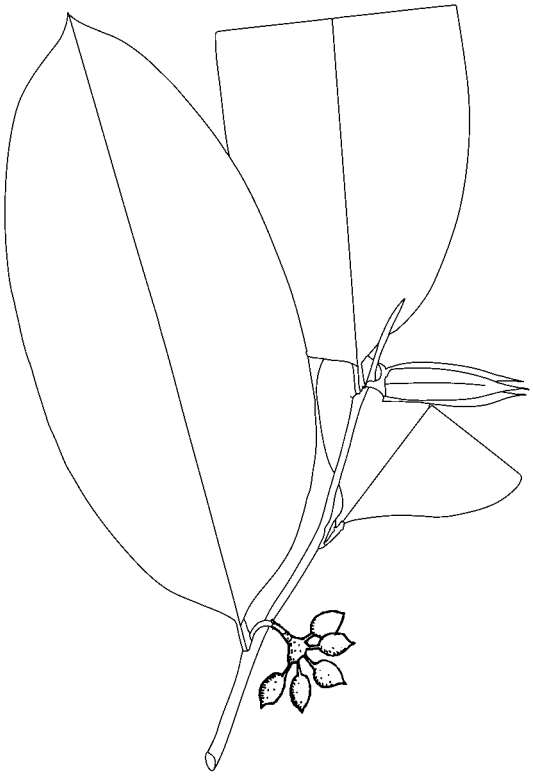
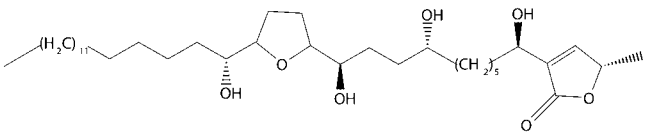


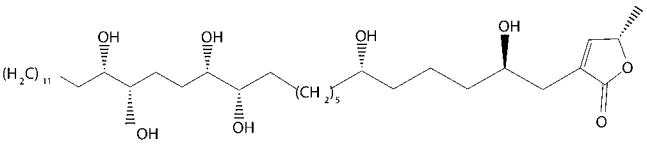
Fig. 103. *Goniothalamus tapis.* From Flora of Malaya. FRI No 19803. Geographical localization: Le Song F.R. Pahang, low undulating seasonal swamp primary forest, alt: 100 feet. Det: Y. C. Chan.

Irrespective of the presence of a large variety of cytotoxic acetogenins and styryl-lactones in the genus *Goniothalamus*, only 19 species out of 160, or 12%, of species have been phytochemically investigated to date, namely *Goniothalamus amuyon* Merr., *Goniothalamus andersonii* J.Sincl., *Goniothalamus arvensis* Scheff, *Goniothalamus borneensis* Mat-Salleh, *Goniothalamus cardiopetalus* Hook. f. & Thoms., *Goniothalamus cheliensis*. Hu, *Goniothalamus donnaiensis* Finet & Gagnep., *Goniothalamus gardneri* Hook. f. & Thoms., *Goniothalamus giganteus* Hook. f. & Thoms, *Goniothalamus griffithii* Hook. f. & Thoms, *Goniothalamus leiocarpus* (W.T.) Wang P.T.Li, *Goniothalamus malayanus* Hook. f. & Thoms, *Goniothalamus montanus* J.Sincl., *Goniothalamus scortechinii* King, *Goniothalamus sesquipedalis Hook. f. & Thoms.*, *Goniothalamus tapis* Miq., *Goniothalamus thwaitesii* Hook. f. & Thoms, *Goniothalamus uvaroides* King., and *Goniothalamus velutinus* Airy Shaw.

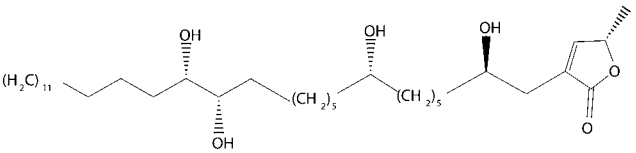
Note that both acetogenins and styryl-lactones are cytotoxic for mammalian cells, as the result of distinct biochemical pathways, which, however, have their molecular origin near or in the mitochondrial membrane and/or the mitochondrial respiratory system (*61*,*62*). Acetogenins were first characterized as the active principles responsible for




Goniotetracin




Gardnerilin A




Gardnerilin B

Fig. 104. Examples of cytotoxic acetogenins from *Goniothalamus* species.

shrimp lethality from the bark of *Goniothalamus giganteus* Hook. f. & Thoms. collected from Thailand. Extract of the bark showed toxicity in the brine shrimp test and showed murine cytotoxicity in the 3PS (P388) leukemia bioassay (*63*).

From the crude ethanol of the bark of *Goniothalamus giganteus* Hook. f. & Thoms., a series of cytotoxic acetogenins were isolated, including giganin, goniothalamicin, annonacin, gigantriocin, gigantetrocin, gigantrionenin, giganenin and gonionenin, goniodenin, asimilobine, gigantecin, bullatalicin, bullatalicinone *cis*-giantrionenine, 4-acetylgigantetrocin A and gigantransenins A, B, and C, pyranicin and pyragonicin, goniotrionin, goniotetracin, and 2,4-*cis*- and *trans*-gonioneninone (*64–71*).

Gigantransenins A, B, and C showed selective inhibitory effects on the human breast tumor cell line (MCF-7) comparable with the potency of adriamycin (*68*). Pyranicin and pyragonicin were selectively cytotoxic against the pancreatic cell line (PACA-2) in a panel of six human solid tumor cell lines, with pyranicin showing 10 times the potency of adriamycin, whereas goniotrionin was more potent against MCF-7. Both goniotetracin and 2,4-*cis*- and *trans*-gonioneninone are selectively and significantly cytotoxic to the human pancreatic tumor cell line (PACA-2).

Goniodonin and 34-*epi*-goniodonins were isolated from *Goniothalamus donnaiensis* Finet & Gagnep. collected from Guangxi Province, China (*72*). Gardnerilins A and B from *Goniothalamus gardneri* Hook. f. & Thoms. collected from DiaoLo mountain, Hainan Province, China, gave cytotoxic $IC_{50}$ values against Bel 7402 human tumor cell lines of 3.6 and 8.5 μg/mL, respectively (Fig. 104; refs. *73–75*).

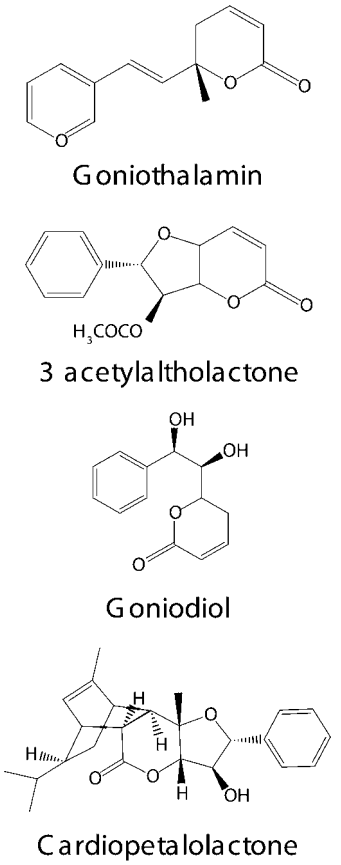



Fig. 105. Examples of cytotoxic styryl-lactones from *Goniothalamus* species.

In regard to the precise molecular mode of action of acetogenins, there is an expanding body of evidence to suggest that both tetrahydrofuran or tetrahydropyran rings and/or hydroxy groups, which are hydrophilic, and the hydrocarbon chains, which are hydrophobic, moor the molecules by anchoring the mitochondrial membrane outside and inside, respectively. The pharmacophores, i.e., the lactones, are therefore secured and interact with the complex I (nicotinamide adenine dinucleotide [NADH]-ubiquinone oxidoreductase) in the mitochondrial electron transport system and abrogate the survival of cancer cells by asphyxiation *(61)*.

Jewers et al. first reported goniothalamin (Fig. 105) as the active constituent of the bark of *Goniothalamus andersonii* J. Sincl., *Goniothalamus macrophyllus* Miq., and *Goniothalamus malayanus* Hook. f. & Thoms. collected in the peat swamp of Sarawak, Malaysia *(75)*. Styryl-lactones such as goniothalamin have since attracted a great deal of interest on account of their ability to inhibit the mitochondrial respiratory chain and to induce apoptosis *(76–83)*. An additional example of an apoptogenic styryl-lactone is altholactone characterized from *Goniothalamus arvensis* Scheff. collected in the

National Park of Variant in the central province of Papua New Guinea, and from the *Goniothalamus borneensis* Mat-Salleh collected in Malaysia (84).

An additional example of a cytotoxic styryl-lactone is cardiopetalolactone characterized from the stem bark of *Goniothalamus cardiopetalus* Hook. f. & Thoms. collected from the Palaruvi forest in Kerala, India, with altholactone, goniopypyrone, cardiobutanolide, goniothalamin, goniodiol, goniofufurone, and goniofupyrone (85,86). 8-Acetylgoniofufurone, 7-acetylgonio-pypyrone, 5-acetylgonio-pypyrone, goniofu-furone, goniopypyrone, goniothalamin, goniothalenol, (+)-isoaltholactone, goniodiol, 7-acetylgoniodiol, goniotriol, 8-acetylgoniotriol, and 9-deoxygoniopypyrone were isolated from the roots of *Goniothalamus griffithii* Hook. f. & Thoms. (87). An isomer of altholactone, (+)-isoaltholactone was isolated from stem bark of *Goniothalamus malayanus* Hook. f. & Thoms, from the leaves of *Goniothalamus montanus* J. Sincl., and the roots of *Goniothalamus tapis* Miq. (88). Goniolactones A through F were identified from the roots of *Goniothalamus cheliensis* Hu, among which was goniolactone B, exhibiting significant cytotoxicity against A2780, HCT-8, and KB cells with $IC_{50}$ values of 7.40, 4.43, and 7.23 μM, respectively (89).

Digoniodiol, deoxygoniopypyrone A, goniofupyrone, goniothalamin, deoxygoniopypyrone A, gonodiol-8-monoacetate, and gonotriol were characterized from the aerial parts of *Goniothalamus amuyon* Merr. collected in the southern part of Taiwan near the coastal regions (90,91). The petroleum ether extract of the stem bark of *Goniothalamus sesquipedalis* Hook. f. & Thoms. collected in Bangladesh afforded goniothalamin, isogoniothalamine oxide, 5-acetoxygoniothalamine, and 5-acetoxygoniothalamine oxide (92). 5-Acetyl goniothalamin was characterized from *Goniothalamus uvaroides* King collected in Bangladesh (93).

Altholactone is apoptogenic in HL-60 promyelocytic leukemia cells via oxidative stress (94). Goniothalamin is cytotoxic against the HepG2 cancer cell line, with $IC_{50}$ values in the range of 0.19 to 0.64 μg/mL, and abrogates the survival of cancerous (HGC-27, MCF-7, PANC-1, HeLa) and non-cancerous (3T3) cell lines time- and dose-dependently (82,95). Goniothalamin induces apoptosis in Jurkat T-cells by the activation of the enzymatic activities of effector caspases-3 and -7 (82). Current paradigms of apoptosis suggest that the loss of mitochondrial transmembrane potential occurs earlier in the commitment phase of apoptosis, which results in the release of mitochondrial apoptogenic proteins—and possibly inhibition of the respiratory chain—including cytochrome *c*, which in the presence of ATP, interacts with Apaf-1, resulting in the activation of caspases 9 (a cysteine proteinase). This, in turn, activates effector caspases such as caspases-3 or -6, hence cell death. Note that styryl-lactones inhibit the mitochondrial respiratory chain in mammalian cells.

A critical factor for *Goniothalamus* use as a medicinal herb is its content of styryl-lactones, which promote apoptosis in mammalian cells. One might set the hypothesis that the abortifacient and/or postnatal and anti-inflammatory traditional uses of *Goniothalamus*

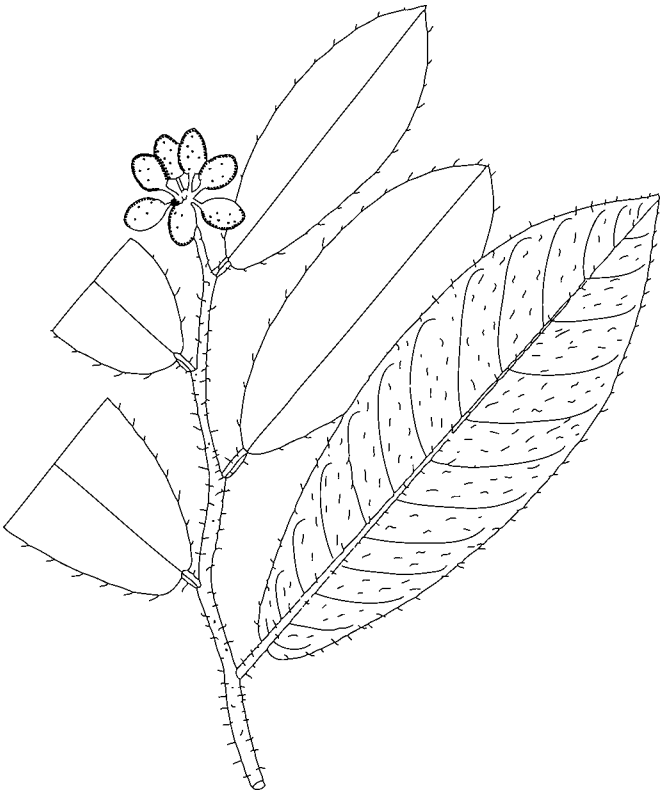
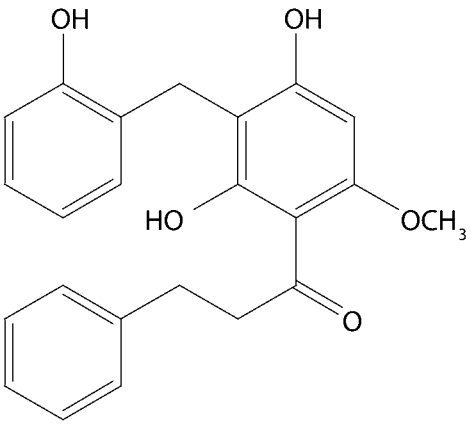


Fig. 106. *Pseudouvaria setosa.* Herb Hort. Bot. Sing. Geographical localization: Raub Pahang. Date: 4/19/1932. Field collector: Osman. Det: J. Sinclair 2/8/1949.

species might involve styryl-lactones because apoptosis is known to play a crucial role in trophoblasts of patients with recurrent spontaneous abortion of unidentified cause, and in T-cells in the human decidua as defense mechanism against rejection of fetal allograft by the maternal immune system (96,97).

***Pseudouvaria setosa* (King) J. Sinclair** (*Orophea setosa* King) is a small tree that grows wild in the primary rainforests of Thailand and the northern part of peninsular Malaysia. The young stems, petioles, leaf margin, lower midrib, and secondary nerves are covered with irritating hairs. The leaves are simple, exstipulate, and alternate and membranaceous, 11–16 cm × 4.5–7 cm. The blade is oblong to oblong-oblanceolate and shows 12–13 pairs of secondary nerves prominent beneath. The flowers are solitary on a 1.8–2-cm-long pedicel. The sepals are minute and suborbicular. The corolla consists of two series of petals which are white with pink bases, ovate, and pubescent. The inner petals are 6 mm long, and united iat apex into a vault. The fruits are one to six globose, ripe carpels that are grayish-yellow and tomentose and up to 2 cm in diameter (Fig. 106).

The roots are chewed by Malays to treat cough. The roots are boiled then reduce into a powder form, which is applied externally to reduce fever. The leaves are part of a mixture eaten with *sireh* to promote libido. The pharmacological properties of *Pseudouvaria setosa* (King) J. Sincl., and of the genus.



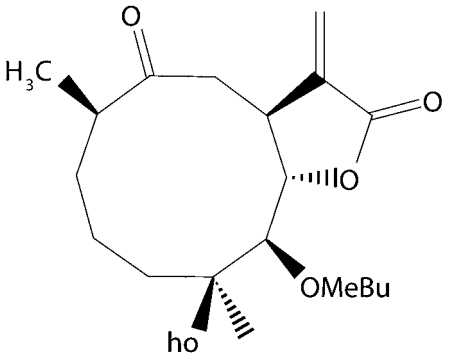

Uvaretin

*Pseudouvaria* in general, is unknown. Note that the *Pseudouvaria* species, and Uvariae in general, have the interesting tendency to elaborate C-benzylated chalcones, which induce apoptosis. Examples of such flavonoids are triuvaretin and isotriuvaretin, uvaretin, isouvaretin, diuvaretin, and angoluvarin from *Uvaria leptocladon* and *Uvaria angolensis* (98–100). Uvaretin and diuvaretin from *Uvaria acuminata* abrogated the survival of human promyelocytic leukemia HL-60 cells cultured in vitro with chromatin degradation and condensation arrest of cells in G1 phase and activation of caspase-3 (101,102).

## Medicinal Asteraceae

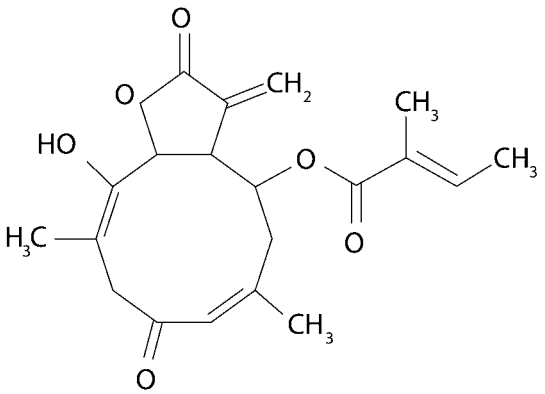
Rivero et al. studied the cytotoxic effects tatridin A, tamirin, reynosin, and ineupatorolide A on the myeloid leukemia cell lines HL-60 and U937, and made the interesting observation that ineupatorolide A, isolated from *Allagopappus viscosissimus*, induced apoptosis accompanied by both the activation of caspase-3 and the fragmentation of poly(ADP-ribose) polymerase-1 and an early release of cytochrome *c* from the mitochondria (103). The evidence for the existence of proapoptotic sesquiterpene lactones in the family Asteraceae is therefore strong, and it seems likely that further study in this field might result in the discovery of antineoplastic agents of clinical value.

An exciting area of potential for proapoptotic agents would be the medicinal flora of the Asia–Pacific region, which encompasses about 250 species of medicinal Asteraceae awaiting pharmacological exploration.




Ineupatorolide A

***Elephantopus mollis* Kunth.** (*Elephantopus cernuus* Vell, *Elephantopus martii* Graham, *Elephantopus scaber* L, *Elephantopus sericeus* Graham, *Elephantopus serratus* Blco.), or elephantopus, elephant's foot, soft elephant's foot, *jangli tambaku* (Fiji), *papago vaca*, *papago halom tano* (Guam), or *lata hina* (Tonga), is an erect herb that grows to a height of 1 m. The plant is native to Central America but has invaded the tropical belt. The stems are pilose, slender, somewhat corymbose, and grayish to bluish-green. The leaves are simple, spiral, grouped in rosette near the roots and cauline along the stem. The blade is pilose, elliptic-oblong or up to 15 cm long, serrate, and lobed. The influorescences are globose heads of minute white or pinkish flowers that are 4 mm long (Fig. 107).




Molephantinin

In Burma, a decoction of the aerial parts is drunk to treat irregular menses. In China, the plant is eaten as a salad and is a believed to improve general health. In several Southeast Asian countries the plant is used to break fevers and prompt urination. In

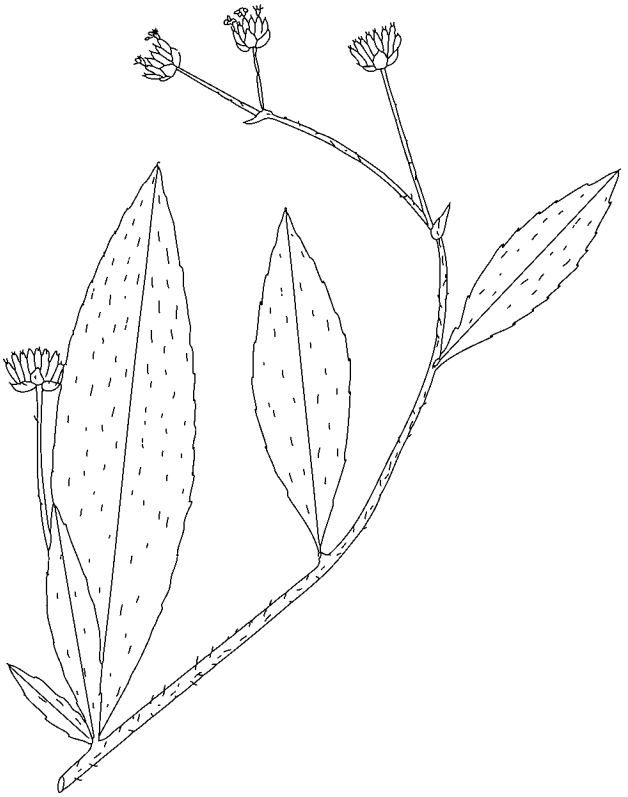


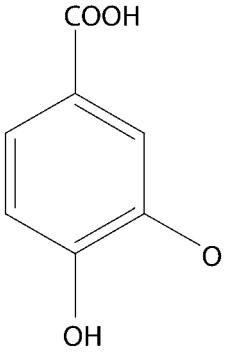
Fig. 107. *Elephantopus mollis.* Flora of Malaya. FRI No 2171. Geographical localization: Fraser's Hill Pahang. Hillside near a stream. 10/15/1966. RM Kochummen. Det: 7/1974, Leiden.

the Philippines, a decoction of the plant provides an emollient remedy. In Guam, the plant is used to treat asthenia fever.

*Elephantopus mollis* is interesting because it elaborates a series of cytotoxic antitumor germacranolides including molephantinin and phantomolin, which are cytotoxic in vitro and in vivo against Ehrlich ascites carcinoma and Walker 256 carcinosarcoma in rodents *(104,105)*. Molephantinin mitigates DNA and protein synthesis in Ehrlich ascites carcinoma cells and DNA synthesis. What is the activity of molephantinin on apoptosis *(106)*?

***Blumea riparia* (Bl.) DC.** (*Blumea pubigera* [L.] Merr., *Blumea chinensis* DC.) is a sprawling herb that grows to 3 ft high in Malaysia, Taiwan, and Indonesia. The stems are terete and finely ribbed. The leaves are simple, spiral, and exstipulate. The blade is lanceolate, elliptic, and serrate and shows five to eight pairs of secondary nerves. The influorescences are conical heads (Fig. 108). In Taiwan, the plant is used externally to assuage headaches. Malays drink a decoction of roots to treat colic. In Indonesia, the plant is used to treat beriberi and gynecological disturbances. The plant is known to contain protocatechuic acid *(107)*.

Fig. 108. *Blumea riparia* (Bl.) DC. Distributed from The Herbarium Botanic Gardens Singapore. No HMB: 2589. 7/17/1961. Coll. & Det.: Burkill. Geographical localization: Gunong Pulai. Exposed on rock. Alt: 1200 ft.




Protocatechuic acid

Kampa et al. made the interesting observation that protocatechuic acid, which is found in grapes and red wine from *Vitis vinifera* (Vitaceae), showed a time- and dose-dependent inhibitory effect on cell growth of T47D human breast cancer cells at low concentrations (*108*). The phenolic compound is a potent inhibitor of topoisomerase I (*109*). The plant likely contains some germanacrolides because these sesquiterpenes are known

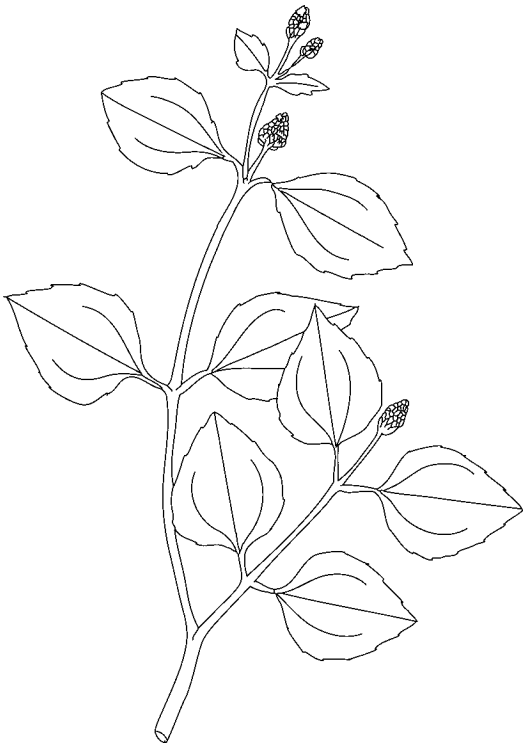


Fig. 109. *Spilanthes paniculata.* From Flora of Singapore, Comm. Ex. Herb. Heort. Bot. Sing. Geographical localization: Geylang Singapore. 7/27/1934. Field collector: Teruya No: 2526. Det. Sri S. Tjitrosvedirjo 7/27/1998.

to occur in members of the genus *Blumea*, the apoptotic property of which is open for exploration *(110)*.

***Spilanthes paniculata* Wall. ex DC** (*Spilanthes acmella* [L.] Murr,), or para cress, toothache plant, *heukala* (Burmese), *pokok getang kerbau*, *kerabu*, *galang*, *gutang* (Malay), *biri* (Philippino), *herbe de Malacca*, *cresson des Indes* (French), or *cuc ao*, *ngo ao* (Vietnamese), is a tropical, branched, annual and herb that grows in the wild to a height of 15–30 cm in open waste places, old clearings, at low and medium altitudes. The stems are glabrous, fleshy, and purplish. The leaves are simple, without stipules, and opposite. The petiole is 2–7 mm long. The blade is 1.5–3 cm long, deltoid, and shows a single pair of secondary nerves. The influorescences consist of conical capitula, which are on 2.5–7-cm-long pedicels. The fruits consist of triquetous or compressed achenes (Fig. 109).

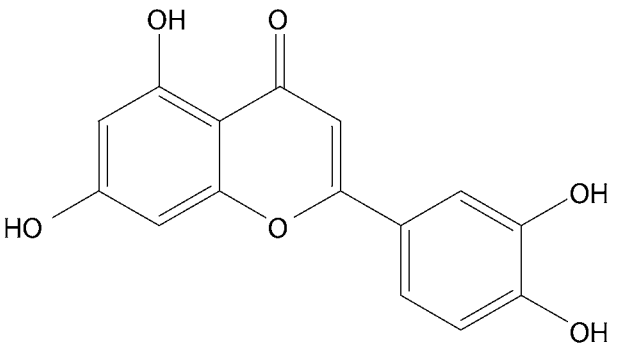
The plant is used in Cambodia, Laos, and Vietnam to treat dysentery and scorbut. The plant is used externally in Malaysia to assuage toothache and headaches, and a decoction is drunk to treat leukemia. In Indonesia, the capitula are chewed to promote salivation. In the Philippines, the roots are used to relieve the bowels from costiveness, and an infusion of the plant is drunk to promote urination. In Papua New Guinea, the

Fig. 110. *Lactula indica.* Flora of Malay Peninsula. Geographical localization: Expt. Plantation Kepong. Date: 6/12/1927. No 11786. Field collector: Ranger.

roots are chewed to mitigate toothaches. It would be interesting to learn whether further experiments on this plant confirm the antileukemic property mentioned previously. Is apoptosis involved here?

***Lactuca indica* L.** (*Lactula brevirostris* Champ.) is an herb that grows in a geographical area spanning from North Asia to Papua New Guinea (Fig. 110). In China, the leaves are eaten as salad to facilitate digestion, and a decoction is drunk to break fever. In Taiwan, the plant is used to break fever, and it is used externally to soothe swellings. In Cambodia, Laos, and Vietnam, the plant is used to invigorate, promote

digestion, and it yields a paste used externally to soothe inflammation. The latex of the plant is known to induce narcosis. In Papua New Guinea, the seeds are chewed for social purposes.




Luteolin

An extract from *Lactuca indica* showed significant free radical scavenging activity, and protected phix174 supercoiled DNA against strand cleavage and reduced oxidative stress in human promyelocytic leukemia HL-60 cells. On account of protocatechulic acid, methyl *p*-hydroxybenzoate, caffeic acid, 3,5-dicaffeoylquinic acid, luteolin 7-*O*-β-glucopyranoside, and quercetin 3-*O*-β-glucopyranoside are the major antioxidative constituents (*111*).

Note that luteolin, a naturally occurring flavonoid, induces apoptosis in various cancer cell lines through death receptor (DR) 5 upregulation. Luteolin induced the expression of DR5, along with Bcl-2-interacting domain cleavage and the activation of caspase-8, -10, -9, and -3. In addition, suppression of DR5 expression with siRNA efficiently limited luteolin-induced caspase activation and apoptosis. Human recombinant DR5/Fc also abrogated luteolin-induced apoptosis (*112*).

## SUMMARY AND FUTURE PROSPECTS

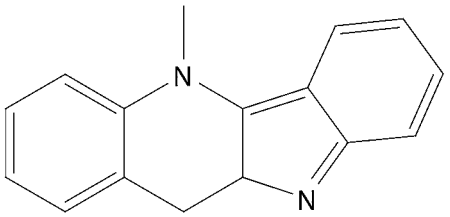
A massive body of evidence has already been presented clearly indicating that the medicinal plants of the Pacific Rim elaborate a broad array of cytotoxic substances. Most of these have been characterized using experimental procedures designed to examine the cytotoxicity of natural products against human tumor cell lines. These procedures involve in vitro screening where the viability of cultured cells after exposure to an extract or a purified substance is measured.

However, one might take the time to think back and to ask if the extensive use of such techniques might not have failed to characterize important lead compounds from plants, and especially medicinal plants. As a matter of fact, a molecule inactive in vitro might, after metabolic transformation in vivo, be effective in abrogating metastasis. The opposite is true, and promising in vitro results have often led to disappointing clinical trials.

In terms of pharmacokinetics, many host factors, such as the route of administration, the metabolism, the catabolism and clearance will considerably determine the antineoplastic success of a drug. One major difficulty with the clinical effectiveness of chemotherapy of neoplastic diseases is the requirement that it kill malignant tumor cells at doses that allow cells in the patient's vital organs to survive so that the recovery can occur. In other words, it is to obtain a reasonably safe therapeutic index favoring introduction into clinical practice.

Ideally, future antineoplastic drug discovery should be based on a more rational, botanical, chemical, and pharmacological approach. A possible way to test the antineoplastic effects of compounds would be to use some semi in vitro–in vivo models. A more rational approach in antineoplastic research, combined with the enormous chemodiversity of flowering plants, will lead to the discovery of several molecules of clinical value.

The ability of natural products to inhibition of topoisomerase and precipitate apoptosis mentioned in this chapter are two abilities among several others, of which inhibition of microtubule formation, inhibition of DNA polymerase, protein kinases, protein phosphatase and aromatase, and the use of cytokines, interleukins, and tumor necrosis factor and yet uncovered cellular targets.

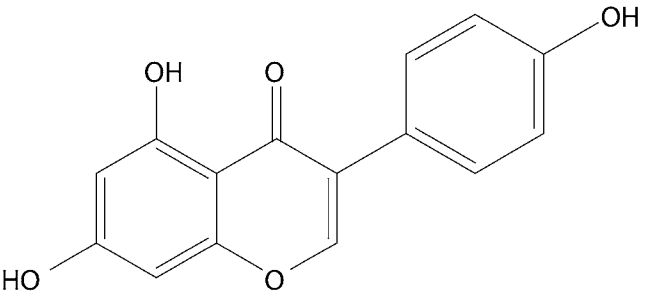



Cryptolepine

An interesting development from the study of the precise pharmacomolecular mechanism of natural products is to bring further light to cellular targets and synergistical cytotoxic mechanisms. One such study has been carried out by Dassonneville et al. They made a careful study of the cytotoxic effects of cryptolepine from the roots of an Asclepiadaceae *Cryptolepis sanguinolenta* and showed that the alkaloids intercalate into DNA and interfere with the enzymatic activity of topoisomerase II, inducing cleavage of poly(ADP-ribose) polymerase and the release of cytochrome *c* from the mitochondria (*113*).

In regard to the inhibitors of tubulin polymerization, Shi et al. showed that a flavonoid such as 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone from a medicinal Capparaceae of the Asia–Pacific region—*Polanisia dodecandra*—abrogated the survival of a surprisingly broad array of cancer cell lines, including central nervous system cancer (SF-268, SF-539, SNB-75, U-251), nonsmall-cell lung cancer (HOP-62, NCI-H266, NCI-H460, NCI-H522), small-cell lung cancer (DMS-114), ovarian cancer (OVCAR-3, SK-OV-3), colon cancer (HCT-116), renal cancer (UO-31), a melanoma

cell line (SK-MEL-5), and leukemia cell lines (HL-60 [TB], SR), and inhibited tubulin polymerization with an $IC_{50}$ value of 0.83 μM (*114*).




Genisteine

An example of flavonoid of interest is genistein (4′5, 7-trihydroxyisoflavone). Perhaps no other flavonoid has aroused more interest in the field of oncology that genistein, a flavonoid found notably in soybean (*Glycine max* [L.] Merr, from the family Fabaceae). Genistein has been suggested to lower the incidence of tumor formation, inhibit protein tyrosine skinase activity, and augment the efficacy of radiation for breast and prostate carcinomas, and underwent phase I/II clinical trials. Several biotechnological firms in Japan, Australia, and in the United States manufacture genistein as food supplement in multimillions of US dollars in yearly benefits.

A definitive conclusion regarding the clinical applicability of natural products would seem premature at this time. In fact, the question remains controversial. The hope for a spectacular cancer cure has not materialized, and there are a few cases where natural products alone seem to yield better results than conventional forms of therapy. The use of a logical approach could bring some change to this rather pessimistic picture. Meanwhile, the medicinal plants of the Asia–Pacific region remain in the stage of "potent source of antineoplastic drugs awaiting discovery."

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