CHAPTER ONE

INTRODUCTION

1.1 Objectives of study

Although chemical agents today remain as the forerunners of insecticidal control of disease-transmitting insects such as mosquitoes, the development of resistance is one of the major concerns in the widespread use of chemical insecticides. As such, the continuous search for new insecticides is a pressing need. Natural products such as rotenone, pyrethrum, nicotine and sabadilla have been used widely as insect repellents and toxicants for a very long time. With the discovery of synthetic insecticides based on chlorinated hydrocarbons, such as DDT, the use of these compounds was greatly reduced for a period of time. However, these chlorinated compounds proved to be either toxic, ecologically unfriendly in the form of bioaccumulation or induced insect resistance. Thus their use has been restricted or banned totally and use of less toxic compounds based on synthetic or natural pyrethroids has been encouraged.

Recently, interest in insecticides of botanical origin has been revived and a large number of plants has been reported to possess insecticidal activity. Neem, *Azadirachta indica* (Meliaceae) a subtropical evergreen native to the arid regions of India has long been used in India for its reputed medicinal and insecticidal properties. Neem oil has exhibited antifeedant activity against *Reticulitermes speratus* and is a potential source of natural pesticide for termite control (Munetaka, *et al.*, 1992). The

active component azadirachtin has been recommended for its unique insecticidal action.

Sarawak's tropical rainforests is a rich source of natural products which have potential medicinal and insecticidal values. The potential source of medicine and insecticides from these and other forests has not been fully exploited despite the concern of continual deforestation.

The objectives of this work are to isolate bioactive larvicidal components from selected Sarawak plants via bioactivity-directed fractionation and to characterise these compounds using spectroscopic methods. This study is focused on plants from the Annonaceae family.

1.2 Botany, distribution and ethnobotany of genera studied

1.2.1 Annonaceae family

The Annonaceae family belongs to the order Ranales (Polycarpicae). The Annonaceae family which consists of five tribes is found throughout the tropics with 120 genera and 2100 species. In the present revision, there are thirty-eight genera, 198 native and five cultivated exotic species making a total of 203 species besides seventeen varieties described in the Malaysian Annonaceae (Kochummen, 1972). Ridley described thirty genera, 180 native species and mentioned four cultivated exotics making a total of 184 species with six varieties (Sinclair , 1955).

Annonaceae are confined mostly to moist tropical lowland forests, in tropical and subtropical countries. None are found in Europe. Fifty-one genera and around 950 species are confined to Asia and Australia, whereas in Africa and Madagascar there are 40 genera and around 450 species. In the American continent, there are thirty-

eight genera and 740 species (Taktajan, 1969; Fries , 1959). The only genus found in the temperate zone is *Asimina* which occurs in Eastern America. Annonaceae are abundant in the Malaysian forests but become scarcer above 2,000 feet elevation. The Malayan Annonaceae are divided into climbing and non-climbing. Their bark is usually smooth and entire, pale grey or buff to brown. Young twigs are pubescent or tomentose, rarely glabrous. They usually become glabrous sooner or later. Plants from the Annonaceae family are readily distinguished from other families by their twigs alone, which often have lozenge-shaped striations resembling a sort of trellis work. The leaves alternate, the flowers are three-merous and the fruits usually are in a group of dry or fleshy carpels attached on a torus (Hsuan, 1983).

Economically, the Annonaceae family is of appreciable importance as a source of edible fruits (Heywood, 1978); the genus *Asimina* provides the paw paw fruit, the *Anona* the sweetsop, soursop, custard apple and ilama. Seeds of some of these plants provide edible oils (Ngiefu *et al.*,1976) and soap (Naidu and Saletore, 1954). Alcohol have also been produced from the woods of Annonaceae plants (Savard and Espil, 1951) and flowers have produced raw materials for perfumery (Klein, 1975). Also, many plants of this family are used for medicinal purposes. The family is not important as a source of commercial timber supplies. Several species have been used for spear shafts and oars. *Mezzetia leptopoda* is utilised for rotary-cut veneers; *Xylopia fusca* is used in making pineapple cases (Kochummen, 1972).

1.2.2 The genus *Goniothalamus*

The genus *Goniothalamus* belongs to the tribe Mitrephoreae (Sinclair, 1955). Plants of the genus *Goniothalamus* are usually shrubs or small trees. The leaves are

usually coriaceous or membranous; the nerves are prominent, oblique, straight and parallel with scalariform reticulations or very fine, sometimes scarcely distinct, not straight or parallel but with a lax network of reticulations, not scalariform. The flowers are usually axillary, sometimes terminal and axillary or cauliflorous. The pedicels have several minute bracts at the base. The sepals valvate, are usually membranous with several veins free or forming a cup and often persistent in fruit. Petals valvate, are leathery, the outer larger than the inner ones. The stamens are many, linear-oblong, the pollen grains large, are visible under a hand lens, the connectives apiculate, flat-topped or convex. Ovaries are numerous, cylindrical, pubescent or glabrous; the style linear and grooved on the anterior side; the stigma are funnel-shaped or narrowly so, split down the inner side, often two-lobed, rarely cylindrical and truncate. The carpels are stalked or sessile; and are 1-2 seeded (four in *Goniothalamus uvarioides*).

There are a total of one hundred and fifteen species (Sinclair, 1955). They are found in South-eastern Asia and throughout Malaysia. The natives of Malaysia find them useful in traditional medicine in connection with childbirth. They are used in attempts to procure abortion as well as to mitigate the violence of the abortient when they are given after childbirth. An undetermined species which is mentioned by Burkill and Haniff (1930) under the name "Kayu bukit" is used after childbirth. The Sakai of Benton use another undetermined species called "Selada" for treating cases where blood is passed in the urine (Shaw,1966).

The timber is often aromatic. The bark is tough and is used for rough ropes in the Philippines and Sumatra. Some of these plants have been used as sources of fibre

(Burkill, 1935; Sastri, 1956) for timber (Burkill, 1935; Watt, 1890), and for ornamental (Corner, 1940) and medicinal purposes (Burkill, 1935; Quisumbing, 1951). Some 29 species are available in Malaysia. They are given in Table 1.1.

Table 1. 1: *Goniothalmus* species available in Malaysia

1. *G. subevenius*	16. *G. calycinus*
2. *G. fulvus*	17. *G. scortechinii*
3. *G. holttumii*	18. *G. montanus*
4. *G. macranii*	19. *G. tapis*
5. *G. giganteus*	20. *G. umbrosus*
6. *G. malayanus*	21. *G. tavoyensis*
7. *G. undulatus*	22. *G. diolichocarpus*
8. *G. tenuifolius*	23. *G. andersonii*
9. *G. rotundisepalus*	24. *G. velutinus*
10. *G. tortilipetalus*	25. *G. nitidus*
11. *G. ridleyi*	26. *G. rufus*
12. *G. macrophyllus*	27. *G. woodii*
13. *G. uvarioides*	28. *G. sinclairinius*
14. *G. wrayi*	29. *G. gigantifolius*
15. *G. curtisii*	

1.2.2.1 *Goniothalamus andersonii*

Goniothalamus andersonii grow in the peat swamps and occasionally in the mixed swamp and Alan Forests[1] of Sarawak. This species is indigenous to Sarawak. *G. andersonii*, known locally as "Lukai amat" by the Ibans in Sarawak, is a tree 10-15 m in height. The leaves are coriaceous or subcoriaceous, oblong, and with length 12-21 cm and breadth 5-8 cm. The bark has a distinct aromatic odour and sharp, bitter taste. The old bark is 8-12 mm thick outer surface light to dark greyish-brown

[1] A narrow forest zone dominated by Alan (*Shorea albida*) but mixed with other dominants of the mixed swamp forest (Anderson , 1980).

Plate 1: Fruits, flowers, leaves (top), and stem (bottom) of *Goniothalamus andersonii*

(Blunden *et al.*, 1973). The bark is usually stripped off from the stem, dried and then burnt to repel mosquitoes.

1.2.2.2 *Goniothalamus dolichocarpus*

Goniothalamus dolichocarpus, a species endemic in Sarawak is a small, slender, treelet growing on clay soils in primary or disturbed lowland forest and at the foot of limestone hills. It grows up to a height of 5 metres and is non-branching. The bark is light brownish-green or greyish-white and is smooth. The inner bark gives a strong smell when cut. The flowers are borne on the stem and are hanging; the fruits are in bundles of a few fruitlets, ripening red (Chai, 1978). The local name is "Lukai bukit". The trees are rare in the Mixed Dipterocarp Forests[2] and grow on alluvial soil. The bark has a very distinctive smell and is used to repel mosquitoes, insects and "evil spirits". The bark is stripped off, dried and burnt. It gives an aromatic smoke which repels insects (Pearce *et al.*, 1987). The Kayans eat the young leaves for stomach ache and diarrhoea and women after giving birth, must carry a piece of burning bark when leaving the house.

1.2.2.3 *Goniothalamus malayanus*

Goniothalamus malayanus is called "Lim panas paya" or "Hujan panas paya". It is frequent in the Mixed Swamp Forest and Alan Forest from the first to the third division in Sarawak. It occurs in the "Kerangas"[3] throughout Sarawak (Anderson, 1980). The stem and root are similar to those of *G. andersonii* except that the young stem is more reddish in colour and the old bark 6 - 8 mm thick, outer surface reddish-

[2] A Mixed Dipterocarp Forest is a very extensive and complex lowland forest on mainly dry soils, but excluding Kerangas forest (Anderson, 1980).

[3] A Kerangas is an inferior forest with mainly small trees, on usually podsolized soils. It is also known as Tropical Heath Forest (Anderson, 1980).

Plate 2: Flower (top), fruits (middle), and leaves (bottom) of *Goniothalamus dolichocarpus*

Plate 3: Fruits and flowers of *Goniothalamus malayanus*

brown with distinct longitudinal wrinkles (Blunden *et al.*, 1974). *Goniothalamus malayanus* is a small tree 4 - 6 m in height. The leaves are glabrous, coriaceous, oblong and with length 13.5 - 21.5 cm and breadth 3.5 - 7.0 cm (Blunden *et al.*, 1973).

1.2.2.4 *Goniothalamus velutinus*

Goniothalamus velutinus is a small tree about 6 m high, is endemic to the island of Borneo. The leaves are coriaceous of length 29.0 - 46.5 cm and breadth 6.5 - 12.5 cm (Blunden *et al.*, 1973). The stem is similar to that of *G. andersonii* except for a dark grey to black outer surface with no distinct longitudinal striations (Blunden *et al.*, 1974). *G. velutinus*, known locally as "Lim panas", is occasionally found in the mixed dipterocarp forests on sandstone substratum and in the "Kerangas" throughout Sarawak. *G. velutinus* has been listed as one of the Bornean medicinal plants (Perry, 1980; Mat Salleh, 1987) although its specific medicinal use is not clearly described. However, the natives in Sabah and Sarawak use its roots decoction for headaches and cases of food poisoning. The leaves which give a strong fragrance are sometimes used as a mosquito repellent (Omar *et al.*, 1992).

1.2.2.5 *Goniothalamus macrophyllus*

Goniothalamus macrophyllus is a shrub or small tree 1.5 - 4.5 m in height. The leaves are glabrous, coriaceous, oblong of length 24 - 44 cm and breadth 7 - 12 cm (Blunden *et al.*, 1973). The young stem and root are similar to those of *G. andersonii* except that the young stem is lighter in colour (Blunden, *et al.*, 1974). The trees are widely distributed in the Mixed Dipterocarp Forests and Submontane

Forests[4] on a variety of soils. They also occur in the "Kerangas" and on limestone (Anderson, 1980). The wood is aromatic. The bark is stripped off the tree, beaten and dried for a few days. It is burnt usually at dawn to drive away "evil spirits". It is used when a member of the longhouse has had a bad omen or a bad dream. "Lukai semeliok" is no longer used by the Christian Ibans (Pearce *et al.*, 1987). A decoction of the roots is used externally for colds and for administering after childbirth (Burkill, 1935).

1.2.2.6 *Goniothalamus ridleyi*

G. ridleyi is a small tree about 3 m tall and 10 cm girth. The bark is stringy with an aromatic smell. It occurs occasionally in the Mixed Dipterocarp Forests on igneous-derived soils and in the peat swamp forests. The local name is "Lukai". The leaves are firmly membranous, the veins are prominent, oblique and parallel. *G. ridleyi* is used to repel mosquitoes and "evil spirits". The bark is stripped off the tree trunk, beaten with a hammer, and then dried. It can be burnt to give a very aromatic smoke which repels mosquitoes and other insects. The smoke from the dried bark is used to drive away honey bees so that the honey comb can be obtained. "Lukai" is especially used at the farm or in the jungle (Pearce *et al.*, 1987).

1.2.2.7 *Goniothalamus uvarioides*

Goniothalamus uvarioides occurs very rarely in the Mixed Dipterocarp Forest on igneous-derived soils (Anderson, 1980).

[4] Forest approximately between 2,000 ft. and 4,000 ft. altitude.

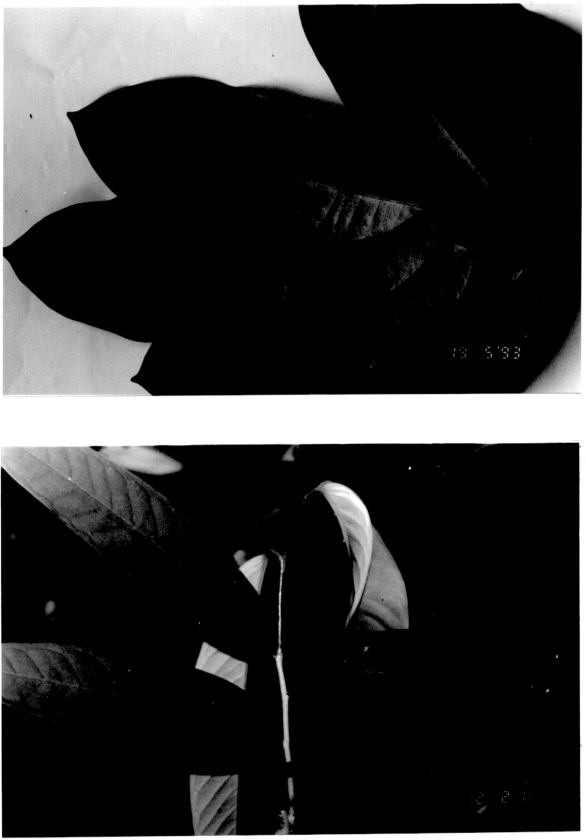

Plate 4: Leaves of *Goniothalamus velutinus* (top), and leaves and shoot of *Goniothalamus macrophyllus* (bottom)

1.2.2.8 *Goniothalamus umbrosus*

Goniothalamus umbrosus is also called "Lukai" locally. The tree is about 2.5 m tall and has a 30 cm girth. It frequents damp "Kerangas" forests and also Mixed Dipterocarp Forests throughout Sarawak. The plant is used as a mosquito and insect repellent. Similarly, the bark is stripped from the tree, completely dried and burnt. It is also extracted for sale (Pearce *et al.*, 1987).

1.2.3 The Genus *Mezzetia*

The genus *Mezzetia* belongs to the tribe Unoneae. The *Mezzetia* are usually tall trees. The leaves are leathery with midrib broad and flat above flushed with upper surface and prominent beneath. The flowers are axillary, small, greenish fasciculate or umbellate. The sepals valvate and are small. The petals also valvate, are spreading, flat, the inner smaller than the outer. The fruits are ellipsoid or globose with hard walls. Seeds are two, large and compressed (Sinclair, 1955). This genus is found in Malaysia. One species, *Mezzetia leptopoda*, which is common in Peninsular Malaysia, has been described as an immense tree. Nothing whatsoever is recorded for its timber. Another species belonging to this genus which is common in southern Sumatra finds its wood useful as planks which are quite durable and useful for protection from rain. The total number of species is seven .

Four species available in Malaysia are:-

1. *Mezzetia leptopoda*
2. *Mezzetia harveyana*
3. *Mezzetia umbellata* and
4. *Mezzetia curtisii*

1.2.3.1 *Mezzetia umbellata*

Mezzetia umbellata is a tall tree reaching ninety feet and has a seven feet girth. *Mezzetia umbellata* is known as "Kepayang babi putih" in Sarawak. It is found occasionally in the Mixed Swamp and Alan Forests and rarely in the Mixed Dipterocarp Forests up to 1500 feet altitude (Anderson, 1980). The twigs are distinctly striate and leaves have 0.2 inch stalks . Leaves are leathery and glaucous below. The midrib is flat and slightly sunk above, raised and sparsely pubescent below. The fruits are elliptic and one inch in size. This species is rare in Selangor and endemic to Borneo (Kochummen, 1972).

1.2.4 The Genus *Disepalum*

The genus *Disepalum* are shrubs or small trees found on mountains. The twigs are reddish-brown. The leaves are glabrous, the margins slightly revolute and the midrib sharply angled on the lower surface. The flowers are bisexual, fragrant, red or yellow tinged with red. The sepals valvate. The petals valvate (Sinclair, 1955; Kochummen, 1972). The fruits are many, ovoid-oblong, and thin-walled. The seeds are 1-2, dark reddish brown and shining. There are six species available in Peninsular Malaya, Borneo and Sumatra. There are two species available in Peninsular Malaya. Some of the available species are:-

1. *Disepalum anomalum*
2. *Disepalum pulchrum* and
3. *Disepalum coronatum*

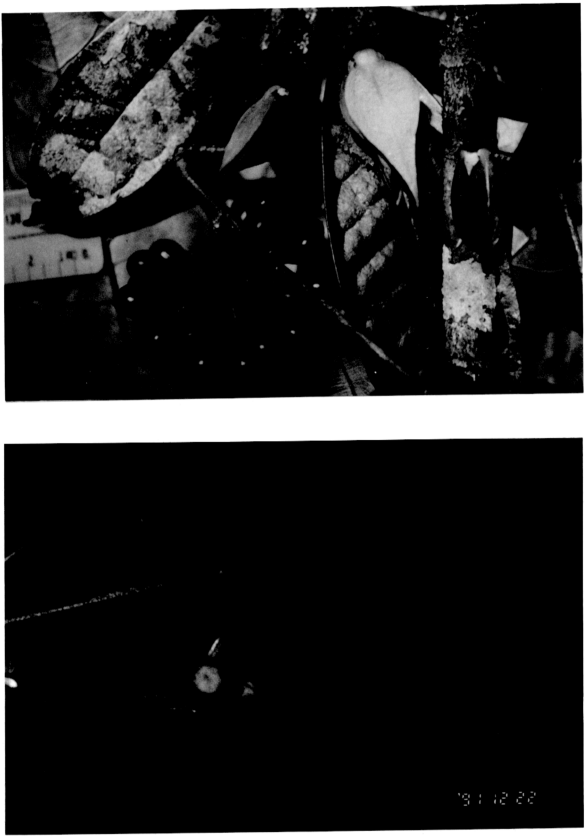

Plate 5: Fruits, leaves and stem of *Goniothalamus umbrosus* (top), Flowers and leaves of *Disepalum anomalum* (bottom)

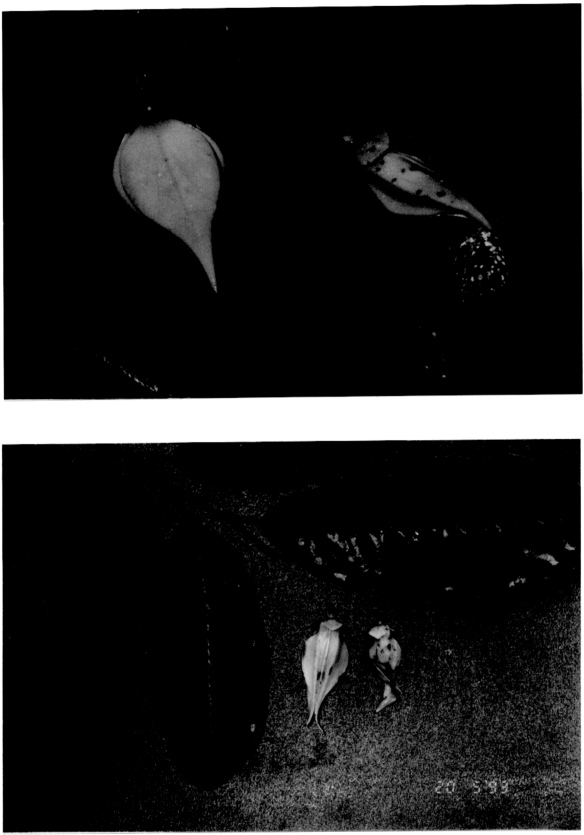

Plate 6: Flowers on branches (top) and leaves (bottom) of *Mezzetia umbellata*

1.2.4.1 *Disepalum anomalum*

Disepalum anomalum is a tree or shrub 5-10 m high and has a 15 cm girth. The bark is brownish-black, hoop marked, somewhat lenticellate. The inner bark is stringy and has a distinct smell. The sapwood is creamy yellow with radiating rays (Pearce *et al.*, 1987). The young twigs are glabrous, dark reddish brown and striate. The leaves are rather similar to those of *Anaxagorea javanica* that is, membraneous, glabrous, oblong to oblong-oborate, and of length 9-10.5 cm and breadth 3-4 cm. This species is available in Perak, Johor, Sarawak and Sabah. In Sarawak, *Disepalum anomalum*, is found rarely in the Mixed Dipterocarp and Alan Forests, and occasionally in the "Kerangas" throughout Sarawak. The bark is used for the construction of chicken coops. The dark outer bark is stripped off, dried and trimmed to the desired size (about 1 m x 3 cm) and then woven into chicken coops of the required shape. The wood is used for house construction. The wood is sawn into roof timbers and joists. This species is used because of its resistance to wood borers. The local name is "Lukai selali".

1.3 Chemistry of the Annonaceae: a literature review

1.3.1 Non-alkaloidal constituents of Annonaceae

Some non-alkaloidal constituents of Annonaceae are polyphenols, essential oils, terpenes, aromatic compounds, carbohydrates, lipids, amino acids and proteins. A large number of studies have been done on the sugars, lipids and proteins contained in the fruits and seeds of certain species of *Annona* of economic importance.

A large number of Annonaceae is fragrant due to the presence of essential oils. The constituents of these oils are usually either well-known monoterpenes and sesquiterpenes or aromatic compounds. The most widely studied essential oil is ylang-ylang oil obtained from the flowers of *Karanga odorata*. It is widely used in perfumery and is hence of considerable economic importance. Seeds of the *Annona squamosa* contain an essential oil comprising α-pinene and caryophyllene. Terpenes of the fruit peel oil are α- and β-pinenes, limonene, β-farnesene, *trans*-ocimene while the leaves contain the terpenes α-pinene, caryophyllene and a cadalenous sesquiterpene (Leboeuf *et al.*, 1982). The fruit of *Xylopia aethiopica* is used in tropical Africa as a substitute for pepper. Its essential oil contains the hydrocarbon β-pinene, cuminal, cineole and terpinen-4-ol as the major constituents (Karawya *et al.*, 1979). A sweet smelling oil obtained from the fruits and seeds of *Dennettia tripetala* yielded a mixture of sesquiterpenes (Okogun and Ekong, 1969).

1.3.1.1 Terpenes and aromatic compounds

Monoterpenes

Camphor and borneol have been found in the roots and bark of *Annona squamosa* (Rao *et al.*, 1978). An investigation of the root bark of *Uvaria chamae* has led to the isolation of a novel C-benzylated monoterpene, chamanen, together with thymoquinol dimethyl ether (Hufford and Lasswell, 1977; Lasswell and Hufford, 1977).

Sesquiterpenes

Leaves of *Annona senegalensis* yielded a sesquiterpene mixture which was reported to possess larvicidal properties (Mackie and Misra, 1956). Bohlmann and

Rao isolated from the roots *of Annona squamosa*, β-caryophyllene accompanied by several kaurane-type diterpenes (Bohlmann and Rao, 1973). Two novel sesquiterpenes yingzhaosu A and yingzhaosu B were isolated from the roots of *Artabotrys uncinatus* (Liang *et al.*, 1979a; Liang *et al.*, 1979b). *Cymbopetalum pendulifiorum* yielded a tetracyclic unoxygenated sesquiterpene, ishwarane (Teng and Debardeleben, 1971). Nitrogenous sesquiterpenes of a special type containing an indole nucleus, have been observed in the genus *Polyalthia.*

Diterpenes

There are about twenty kaurane diterpenes which have been reported from several *Annona* species and *Xylopia aethiopica.* This includes stachanoic acid (the stachnane skeleton is closely related to that of kaurane). The structure of xylopic acid initially isolated by Ekong and Ogan and tentatively assigned (Osman *et al.*, 1971) was discarded after the structure was confirmed by X-ray analysis (Fiagbe *et al.*, 1979). Xylopic acid was found to have antimicrobial properties (Boakye-Yiadom *et al.*, 1977). A new diterpene acid, polyalthic acid, with a labdane skeleton was isolated from the stem bark of *Polyalthia fragrans* (Gopinath *et al.*, 1961).

Triterpenes, sterols and saponins

Phytochemical screening tests have shown the presence of sterols and saponins in various genera of the Annonaceae family. Sitosterol have been frequently isolated from the leaves of *Annona muricata* (Callan and Tutin, 1911) and from the seeds, roots and bark of several *Annona* species. Other sterols isolated are stigmasterol, campesterol and cholesterol .

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Aromatic compounds

From the root bark of *Uvaria chamae* and the stem bark of *Uvaria ovata*, various aromatic compounds were isolated (Okorie, 1977; Hufford and Lasswell, 1977a; Lasswell and Hufford, 1977b). They were benzyl benzoate, *o*-methoxybenzyl benzoate, *o*-methoxybenzyl ether and di-*o*-methoxybenzyl ether. Several propenylbenzene and vinylbenzene derivatives have been recorded from annonaceous plants.

1.3.2 Alkaloids of Annonaceae

Almost all the alkaloids isolated from the Annonaceae family possess an isoquinoline-derived structure. They are mainly simple isoquinolines, benzyltetrahydro-isoquinolines, protoberberines, aporphinoids, including aporphines, oxoaporphines, phenanthrenes and miscellaneous isoquinoline-type alkaloids.

Oxoaporphines

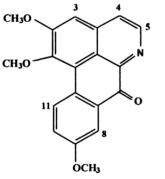
Oxoaporphines have been isolated from various annonaceous plants, liriodenine being ubiquitous. From the structural point of view, these oxoaporphines are rarely substituted at positions 3, 9, 10 and 11. Oxostephanine is a rare example of an 8-substituted aporphine.

A new botanical source of oxoaporphines is the family Eupomatiaceae (Bowden *et al.*, 1975). Some recently identified oxoaporphines are shown in Table 1.2 and their structures shown below.

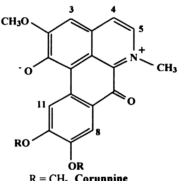
Table 1. 2: Occurrence of oxoaporphines in Annonaceae

Alkaloid	Source
Atherospermidine	*Enantia polycarpa* *Guatteria psilopus*
Lanuginosine (oxoxylopine)	*Annona squamosa* *Enantia pilosa* *Polyalthia emarginata* *Polyalthia oliveri* *Xylopia brasiliensis* *Xylopia buxifolia* *Xylopia lemurica*
Liriodenine	*Annona cherimolia* *Annona glabra* *Annona montana* *Annona reticulata* *Annona squamosa* *Asimina triloba* *Cananga latifolia* *Canaga odorata* *Enatia pilosa* *Enantia polycarpa* *Fusaea longifolia* *Guatteria modesta* *Isolona campanulata* *Melodorum punctulatum* *Mitrella kentii* *Pachypodanthium staudtii* *Polyalthia emarginata* *Polyalthia nitidissima* *Polyalthia oliveri* *Pseuduvaria* sp. *Pseuduvaria* cv. *grandifolia* *Schefferomitra subaequalis* *Uvariopsis guineensis* *Xylopia brasiliensis* *Xylopia buxifolia* *Xylopia pancheri* *Xylapia vielana*
Lysicamine (oxonuciferine)	*Enantia chlorantha* *Enantia polycarpa* *Polyalthia suaveolens*
O-Methylmoschatoline	*Duguetia eximia* *Enantia chlorantha* *Guattera subsessilis*
Oxoanolobine	*Guatteria melosma*
Oxoglaucine (*O*-methylatheroline)	*Annona purpurea*
Oxolaureline (lauterine)	*Guatteria elata*
Oxopukateine	*Duguetia eximia*
Oxopurpureine	*Annona purpurea*
Oxoputerine	*Duguetia calcyina* *Duguetia eximia* *Guatteria elata*
Oxosterphanine	*Polyalthia sauveolens*
Subsessiline	*Guatteria subsessilis*
Noname (compound D)	*Guatteria melosma*

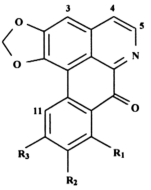
Source: Leboeuf *et al.*, (1982).



Subsessiline
(Hasegawa *et al.*, 1972)



R = CH_3, **Corunnine**
R + R = CH_2, **Nandazurine**
(Kunitomo *et al.*,1974)

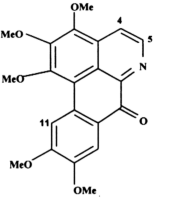


R_1 = OCH_3, R_2 = R_3 = H,
Oxostephanine
(Watanabe *et al.*, 1975)

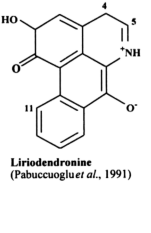
R_1 = R_2 = R_3 = H,
Liriodenine (Ferdous *et al.*, 1992)

R_1 = R_3 = H, R_2 = OMe,
Lanuginosine
(Ferdous *et al.*, 1992)

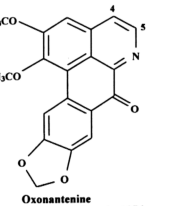
R_1 = R_2 = H, R_3 = OMe,
Oxolaureline (Urzua and Cassels, 1976)



Oxopurpureine
(Martinez *et al.*, 1988)



Liriodendronine
(Pabuccuoglu *et al.*, 1991)



Oxonantenine
(Urzua and Cassels, 1976; Hsu *et al*, 1977)

Figure 1. 1: Some recently identified oxoaporphines

Careful analysis of the ^1H NMR of oxolaureline has shown that in oxoaporphines H-11 does not necessarily appear downfield from H-8 as has been generally assumed. For this alkaloid both H-5 and H-8 absorb further downfield than H-11 regardless of whether the solvent used is TFA or $CDCl_3$ (Urzua and Cassels, 1976; Hsu *et al.*, 1977). With oxonantenine, H-11 is furthest downfield when using TFA, but is upfield from H-5 when $CDCl_3$ is the solvent (Urzua and Cassels, 1976).

4,5 - Dioxoaporphine

It is only recently that the reddish orange 4,5-dioxoaporphines have been recognized as a distinct group of isoquinoline alkaloids. This group includes cepharadione-A and -B, norcepharadione B and pontevedrine which was previously believed to be a 5,7-dioxoaporphine. 4,5-Dioxoaporphines clearly originate biogenetically from oxidation of aporphines. 4,5-dioxoaporphines generally occur in the genera Menispermaceae, Papaveraceae and Piperaceae. The 4,5-dioxoaporphines (1-demethoxy-4,5-dioxodehydroasimilobine and 4,5-dioxodehydroasimilobine) have recently been isolated from *Monocylclanthus vignei* of the Annonaceae family.

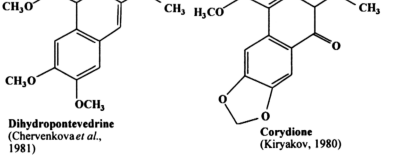
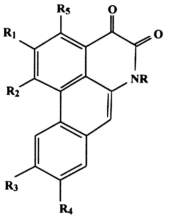


Figure 1. 2: Structures of dihydropontevedrine and corydione



$R_1 = R_2 = OMe$, $R_3 = R_4 = R_5 = H$, R = Me,**Cepharadione B** (Achenbach *et al.*, 1991; Desai *et al.*, 1988)

$R_1 = R_2 = R_3 = R_4 = OMe$, R = Me, $R_5 = H$ **Pontevedrine** (Castedo *et al.*, 1976)

$R_1 = R_2 = OMe$, $R_3 = R_4 = R_5 = R = H$ **Norcepharadione B** (Achenbach *et al.*, 1991; Desai *et al.*, 1988)

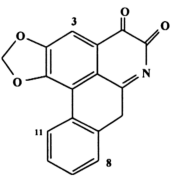
$R_1 = R_2 = R_5 = OMe$, $R_3 = R_4 = H$, R = Me, **3 - Methoxycepharadione B** (Mahmood *et al.*, 1986)

$R_1 = OH$, $R_2 = OMe$, $R_3 = R_4 = R_5 = R = H$, **4,5 - Dioxodehydroasimilobine** (Noraristolodione) (Achenbach *et al.*, 1991)

$R_1 = OH$, $R_2 = OMe$, $R_3 = R_4 = R_5 = H$, R = Me **Aristolodione** (Urzua and Cassels, 1987)

$R_1 = R_2 = R_5 = OMe$, $R_3 = R_4 = R = H$, **Ouregidione**, (Cortes *et al.*, 1986)

$R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = R = H$, **6a,7-Dehydro-2-hydroxy-4,5,-dioxonoraporphine** (Achenbach *et al.*, 1991)



Cepharadione A (Achenbach *et al.*, 1991)

Figure 1. 3: Structures of some 4,5-dioxoaporphines

Pontevedrine originally assigned as 5,7-dioxoaporphine has had its structure revised to the 4,5-dioxoaporphine after it was discovered that (+)-cataline also isolated from the same source could be easily oxidised to pontevedrine using iodine or DDQ (dichlorodicyanoquinone). This suggested a new formula for pontevedrine namely, 4,5-dioxoaporphine (Figure 1.4).

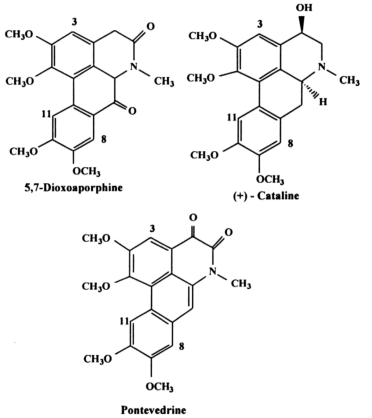


Figure 1. 4: Originally assigned and revised structures for pontevedrine

Treatment of pontevedrine with sodium hydroxide in methanol gave the yellow aristolactam through the intermediate α-hydroxyacid anion which was not isolated (Castedo *et al.*,1976) (Figure 1.5).

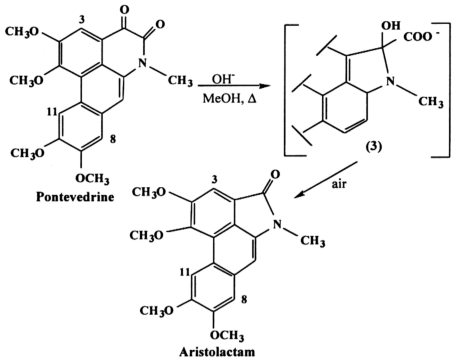


Figure 1. 5: Synthesis of aristolactam

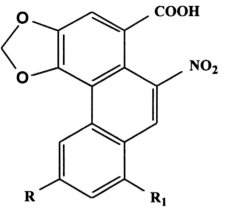
Benzilic acid rearrangements within this context may have some analogy in nature, since aristolactams are found as natural products.

The orange coloured cepharadione-A and -B found in *Stephania cepharantha* (Menispermaceae) were the first 4,5-dioxoaporphines to be fully characterised.

Aristolochic acid and aristolactams

Aristolochic acids and aristolactams occur mostly within the family of Aristolochiaceae although cepharanone-A and aristolactam B-II were found in *Stephania cepharantha* (Menispermaceae) (Akasu *et al.*, 1974) and doryflavine was obtained from *Doryphora sassafras* (Monimiaceae) (Chen *et al.*, 1974). Six aristolochic acids and twelve aristolactams are shown below (Figures 1.6 and 1.7).

Aristolochic acids



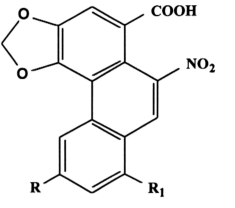
R = H, R_1 = OCH_3
Aristolochic acid I
(Kupchan and Wormser, 1965)

R = OH, R_1 = H
Aristolochic acid C
(Sasagawa, 1959;
Tomita and Sasagawa, 1959)

R = OH, R_1 = OCH_3
Aristolochic acid D
(Kupchan and Merianos, 1968)

Aristolochic acid IVa
(Ruveda *et al.*, 1968)

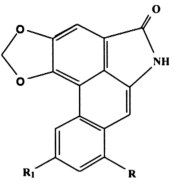
R = H, R_1 = H
Aristolochic acid II
(Pailer and Schleppnik, 1958)



R = OH, R_1 = H
Aristolochic acid B
(Sasagawa 1959;
Tomita and Sasagawa, 1959)

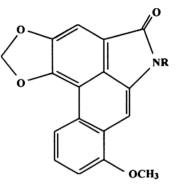
Figure 1. 6: Structures of some aristolochic acids

Aristolactams



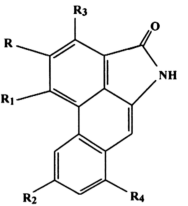
R = H, R_1 = H,
Aristolactam
(Sasagawa, 1959;
Tomita and Sasagawa, 1959)

R = OCH_3, R_1 = OCH_3,
Aristolochic acid D
methyl ether lactam
(Kupchan and Merianos, 1968)



R = H **Aristored**
(Pailer *et al.*, 1956;
Coutts *et al.*, 1957)

R = β-glucoside,
Aristolactam β-D-glucoside
(Kupchan and Merianos, 1968)



R = R_1 = R_4 = OCH_3,
R_2 = R_3= H,
Taliscanine (Maldonado *et al.*, 1966)

R = R_1 = R_2 = OCH_3, R_3 = R_4 = H,
Aristolactam BIII
(Crohare *et al.*, 1974)

R = OH, R_1 = OCH_3,
R_2 = R_3 = R_4= H,
Aristolactam AII (Crohare *et al.*, 1974)

R = OCH_3, R_1 = OH,
R_2 = R_3 = R_4= H,
Doryflavine (Chen *et al.*, 1974)

R = OH, R_1 = OCH_3,
R_2 = OCH_3, R_3 = R_4 = H,
Aristolactam AIII (Crohare *et al.*, 1974)

R = OH, R_1 = R_3 = OCH_3,
R_2 = R_4 = H,
Goniopedaline (Talapatra *et al.*, 1988)

R = R_1 = OCH_3, R_2 = R_3 = R_4 = H,
Aristolactam BII or Cepharone B
(Crohare *et al.*, 1974)

R = R_1 = OCH_3,
R_2 = R_3 = R_4= H,
Velutinam (Omar *et al.*, 1992)

Figure 1. 7: Structures of some aristolactams

The following scheme shows the main degradation sequences of aristolochic acid-I into phenanthrene and aristolactam.

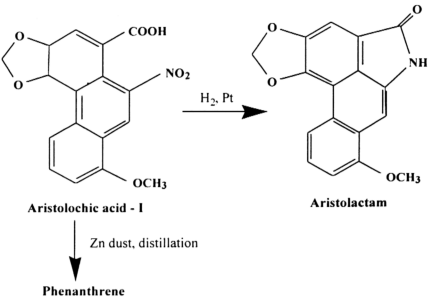


Figure 1. 8: Degradation sequences of aristolochic acid-I into phenanthrene and aristolactam

There is a possibility that a 1,2,4-trioxygenated aporphine is oxidised to a 4,5-dioxoaporpine such as cepharadione-A which can undergo net overall decarbonylation, presumably through a benzilic acid rearrangement, to yield the corresponding aristolactam cepharanone A, in this case. Further oxidation would then yield aristolochic acid-II (Shamma and Moniot, 1976; Castedo *et al.*, 1976)). Cepharadione-A and cepharanone-A occur in the same plant, *Stephania cepharantha* (Menispermaceae) (Akasu *et al.*, 1974).

It is also observed that aristolochic acid and aristolactams are never found in nature oxygenated at C-7. Five phenanthrene derivatives (see Figure 1.9) have been

isolated from *Aristolochia indica*. All five must be of aporphinoid origin (Pakrashi *et al.*, 1977).

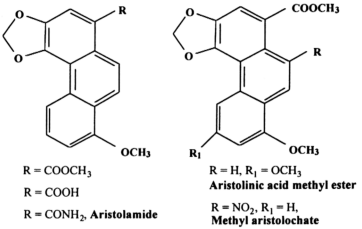


Figure 1. 9: Structures of five phenanthrene derivatives

More recently twelve aristolactam alkaloids were isolated from *Aristolochia argentina*, four of which contains hydroxymethyl and carboxyl groups as substituents (Priestap, 1985) (see Figures 1.9 and 1.10).

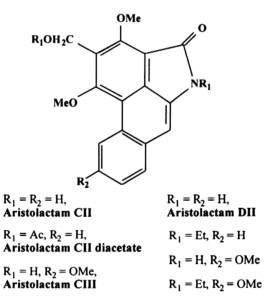
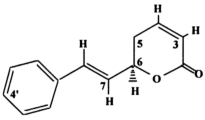


Figure 1. 10: Structures of some aristolactam alkaloids

1.3.3 Styrylpyrones of the genus *Goniothalamus*

1.3.3.1 Goniothalamin

Several *Goniothalamus* species have provided a number of styrylpyrone derivatives of which (+)-goniothalamin is usually dominant. (+)-Goniothalamin was originally isolated from *Cryptocarya caloneura* (Hlubucek and Robertson, 1967) and *Goniothalamus andersonii* (Jewers *et al.*, 1972). (+)-Goniothalamin was originally assigned the (*6S*)-configuration based on a degradation study (Hlubucek, 1967) but was revised later to the (*6R*)-configuration on the basis of synthetic studies (Meyer, 1979; O'Connor and Just, 1986; Bennet and Knight, 1988; Honda *et al.*, 1990).



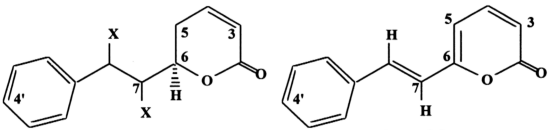
(+)-Goniothalamin

Figure 1. 11: Structure of (+)-goniothalamin

Despite this, a recent paper (Sam *et al.*,1987) quoted the (*6S*)-configuration for this compound. Also the absolute stereochemistries of a series of reported oxygenated goniothalamin homologues are in need of revision (Sam *et al.*, 1987; Ahmad *et al.*, 1991; Wu *et al.*, 1992; Talapatra *et al.*,1985). Hence the previously isolated goniothalamin-7,8-epoxide (Sam *et al.*, 1991) has the *6R,7R,8R* configuration rather than *6S,7R,8R* assignment which was given on the basis of goniothalamin having a (*6S*)-configuration.

In addition, this supports the original absolute configuration assigned to the biosynthetically related tetrahydrofuran-2-pyrone, goniothalenol (El-Zayat *et al.*, 1985) rather than the enantiomeric structure mentioned by Sam *et al* (1987).

Treatment of goniothalamin with NBS gave three products which could be partially separated by trituration with ether (Jewers *et al.*, 1972). IR spectrum of the major component showed strong absorption at 1730 cm^{-1} indicating that the molecule still contained an α,β-unsaturated lactone moiety. Proton NMR indicated the absence of styryl olefinic protons. This indicated that bromine addition had occurred at the styryl olefinic protons and that the conformation of the side chain is such that H-7 and H-8 is *trans*-diaxial to give the *erythro*-7,8-dibromogoniothalamin. The minor component of the ether insoluble fraction was shown to be *threo*-7,8-dibromogoniothalamin. Chromatographic separation of the ether soluble portion of the reaction products derived from the above some reaction afforded 6-styryl-2-pyrone (see Figure 1.12).



***Threo* and *erythro*-7,8-dibromogoniothalamin**

Figure 1. 12: Structures of *threo* and *erythro*-7,8-dibromogoniothalamin

Styryl-2-pyrones have also previously been reported as constituents of the Lauraceae (Bittencourt *et al.*, 1971; Gottlieb, 1967), Piperaceae (Achenbach and Whittman, 1970; Hansel, 1968) and Basidiomycetes (Hatfield and Brady, 1970).

(+)-Goniothalamin is biologically active and found to have antifungal and CNS activity. Stem bark, stem wood, leaves, root bark, root wood and fruits of four species of *Goniothalamus* were assayed for goniothalamin. Goniothalamin was detected in all parts tested on *G. andersonii* and *G. macrophyllus* and in some samples of *G. malayanus* but was not detected in some parts of *G. velutinus* samples (see Table 1.3) (Jewers *et al.*, 1972).

Table 1. 3: Goniothalamin contents in different morphological parts of *Goniothalamus* species

Species	Part	Goniothalamin yield (% air-dried wt.) for sample:			
		A	B	C	D
G. andersoni	Stem bark	2.10	0.63	0.49	-
	Stem wood		1.36	0.53	
	Young stems	0.25			
	Leaves	+	+	+	
	Root bark			0.75	
	Whole root	3.00			
	Fruit	6.10			
G. macrophyllus	Stem bark	0.95			
	Stem wood	1.52			
	Leaf	+			
	Root bark	0.68			
	Root wood	0.83			
G. malayanus	Stem bark		-	0.22	-
	Stem wood		-		
	Young stems	0.05			
	Leaves		-		
	Root bark		+		
	Root wood		-		
G. velutinus	Stem bark	-	-		
	Stem wood	-	-		
	Leaves	-	-		

Source: Jewers *et al.*, (1972). + denotes positive for goniothamalin, but very low yield. - denotes goniothalamin not detected.

(+)-*6R*-Goniothalamin could be synthesised from the olefinic aldehyde by witting reaction (O'Connor and Just, 1986) (see Figure 1.13). Later on Honda *et al.*, (1990) was able to synthesise the same compound from 2-furylmethanol as the starting material. The synthetic strategy was based on the kinetic resolution of racemic secondary 2-furylmethanols by using the Sharpless reagent and making the assumption that pyranones with high optical purity could be obtained when the reaction was carried out only to some degree of conversion below 50% (Kametani *et al.*, 1988; Kusakabe *et al.*, 1989). With this assumption, the optically active pyranone was prepared to be used as the key intermediate for the synthesis of (+)-6*R*-goniothalamin (Figure 1.13).

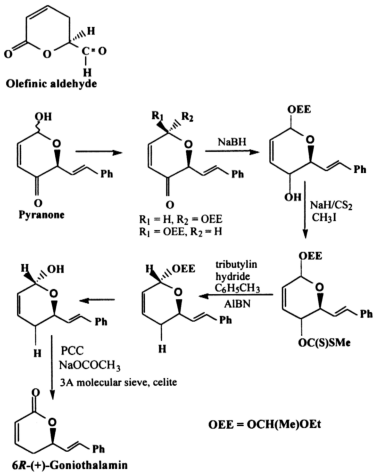


Figure 1. 13: Synthesis of *6R*-(+)-goniothalamin

Earlier on, Bennet and Knight (1988) had synthesised both enantiomers of goniothalamin. Dehydration of the *trans*-lactone with $POCl_3$ in pyridine at 70 °C for 1 hour gave (*6S*)-goniothalamin which was identical in all respects with natural goniothalamin except for the optical rotation ($[\alpha]_D^{20}$ = -130 [c 0.7, $CHCl_3$]). (*6R*)-Goniothalamin could be obtained from the *cis*-lactone under the same reaction conditions. This compound is identical in all respects with the natural product including optical rotation ($[\alpha]_D^{20}$ + 126 [c 0.5, $CHCl_3$]) (see Figure 1.14).

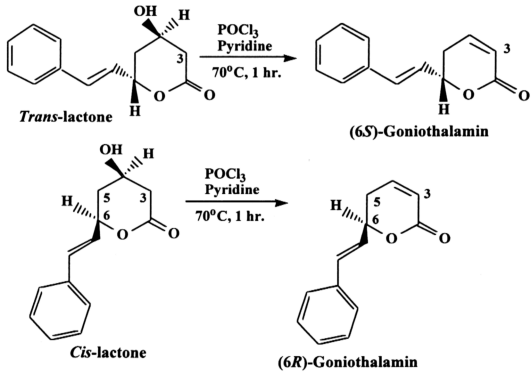


Figure 1. 14: Syntheses of (*6S*)-goniothalamin and (*6R*)-goniothalamin

Phytochemical studies of some other *Goniothalamus* species have resulted in the isolation of a number of styrylpyrone derivatives (Figure 1.15). Talapatra *et al.*,

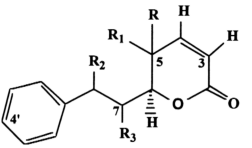
(1985) isolated four styrylpyrones from *Goniothalamus sesquipedalis* and *G. grifithii*. The leaves and twigs of *G. sesquipedalis* gave goniodiol diacetate [6-(1,8-dihydro-18,8-diacetoxystyryl)-5,6-dihydro-2-pyrone], goniodiol monoacetate [6-(7,8-dihydro-7-acetoxy-8-hydroxystyryl)-5,6-dihydro-2-pyrone, goniodiol [6-(7,8-dihydro-7,8-dihydroxystyryl)-5,6-dihydro-2-pyrone] and goniotriol [6-(7,8-dihydro-7,8-dihydroxystyryl)-5,6-dihydro-5-hydroxy-2-pyrone]. However, no absolute configurations for the compounds were reported. Later on, the same goniotriol was isolated by Alkofahi and the absolute configuration reported (Alkofahi *et al.*, 1989). The compound goniodiol-7-monoacetate was also later isolated from *Goniothalamus amuyon* and its crystal structure and cytotoxicity reported (Wu *et al.*, 1991).

The novel tetrahydrofurano-2-pyrone ring system compound, goniothalenol was isolated from *Goniothalamus giganteus* by McLaughlin and co-workers (El-zayat *et al.*, 1985). This work reported the bioactivity-directed fractionation of the stem bark extract using the brine shrimp lethality test. The stereoisomer of this compound (+)-isoaltholactone was isolated from the stem bark of *Goniothalamus malayanus*, the stem bark and leaves of *G. montanus* and the roots of *G. tapis* from Malaysia. The relative and absolute configurations of goniothalenol and isoaltholactone, respectively were determined (Colegate *et al.*,1990). *Goniothalamus macrophyllus* from Malaysia furnished the active embryotoxic and teratogenic components goniothalamin and goniothalamin epoxide (Sam *et al.*, 1987). However, this paper quoted the (6*S*)-configuration for goniothalmin epoxide which is indeed in need of revision.

Fang and co-workers reported two new styrylpyrone derivatives goniopypyrone and goniofufurone from *G. giganteus* (Fang *et al.*, 1990). The same

group of researchers later isolated the derivative of goniofufurone 7-*epi*-goniofufurone along with the derivative of goniopypyrone 9-deoxygoniopypyrone from the same plant (Fang *et al.*, 1991). The relative configurations of 7-*epi*-goniofufurone and 9-deoxygoniopypyrone were reported in this work. In the same work, the reported natural product, goniodiol was isolated as a waxy oil instead of the needle-like crystals as reported previously by Talapatra *et al.*, (1985). At the same time, the new styrylpyrone derivative 5-acetoxygoniothalamin was reported to be isolated from *Goniothalamus uvaroides* of Sabah, East Malaysia (Ahmad *et al.*,1991).

The same *Goniothalamus amuyon* gave the cytotoxic goniodiol 8-monoacetate [6*R*-(7*R*,8*R*-dihydro-7-hydroxy-8-acetoxystyryl)-5,6-dihydro-2-pyrone] together with goniotriol a year after goniodiol 7-monoacetate was discovered (Wu *et al.*, 1992). For the first time, the epoxystyryl lactone, 5-acetoxyisogoniothalamin oxide was reported to be isolated from *Goniothalamus sesquipedalis* from Bangladesh. This plant had previously furnished three phenanthrene lactams. The new epoxystyryl lactone was assigned as *6S,7S,8R* (Hassan *et al.*, 1994). We isolated the new styrylpyrone derivatives (-)-iso-5-deoxygoniopypyrone (Goh *et al.*, 1995a) and (+)-5β-hydroxygoniothalamin (Goh *et al.*, 1995b) both of which came from the same plant *Goniothalamus dolichocarpus*. Very much later, a new styryl-lactone (+)-goniotharvensin was isolated from the *Goniothalamus arvensis* from Papua, New Guinea. This compound is very much the same as goniothalenol except for the absence of a double bond at the C-3 and C-4 position (Bermejo *et al.*, 1995).



R = R_1 = H,
R_2 = OH, R_3 = OAC,
Goniodiol-7-monoacetate

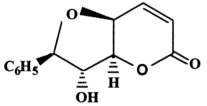
R = R_1 = H,
R_3 = OH, R_2 = OAC,
Goniodiol -8-monoacetate

R_1 = H, R = R_3 = OH,
R_2 = OAc,
8-Acetylgoniotriol

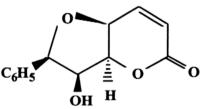
R = R_2 = R_3 = OH,
R_1 = H, Goniotriol

R = R_1 = H, R_2 = R_3 = OAC,
Goniodiol diacetate

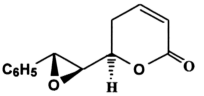
R = R_1 =H, R_2 = R_3 = OH,
Goniodiol



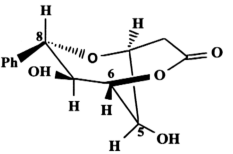
(+)-Goniothalenol



(+)-Isoaltholactone

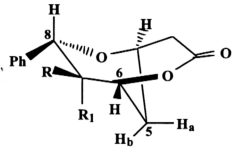


(+)-Goniothalamin epoxide



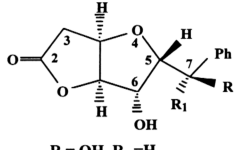
(+)-Goniopypyrone

Figure 1. 15: Structures of some styrylpyrone derivatives



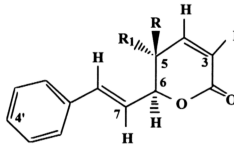
R = H, R_1 = OH,
(+)-5-Deoxygoniopypyrone

R = OH, R_1 = H,
(-)-Iso-5-deoxygoniopypyrone



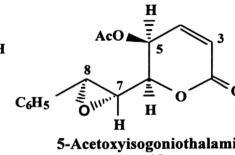
R = OH, R_1 =H,
Goniofufurone

R = H, R_1 = OH,
7-Epi-Goniofufurone

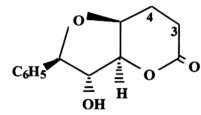


R = OAc, R_1 = H,
(+)-5-Acetoxygoniothalamin

R = OH, R_1 = H,
(+)-5β-Hydroxygoniothalamin



5-Acetoxyisogoniothalamin
Epoxide



(+)-Goniotharvensin

Figure 1.15: Structures of some styrylpyrone derivatives (continued)

1.3.4 Annonaceous acetogenins from the family Annonaceae

Acetogenins from Annonaceae are a series of C_{35}-C_{39} natural products derived from fatty acids. They typically contain two long hydrocarbon chains with a terminal 2,4-disubstituted-γ-lactone ring and a variable number of tetrahyrofuran (THF) rings. The hydrocarbon chains contain several oxygenated substituents which can be hydroxyls, acetoxyls and/or ketones. Recently, single ring acetogenins containing

double bonds, epoxide compounds which lack THF rings and a compound lacking both epoxides and THF rings have been reported. So far, all the acetogenins which have been isolated contain multiple stereocentres. The acetogenins are of waxy nature, hence do not produce crystals suitable for X-ray crystallographic analysis. Relative stereochemistries of ring junctions are typically determined by comparing the natural products with synthetic model compounds using high field nuclear magnetic resonance analysis. Their methoxyfluoromethylphenylacetic acid (MPTA) esters or more commonly known as Mosher esters are prepared and analysed by high field NMR. Most novel acetogenins have been isolated only from the Annonaceae. These acetogenins are potently bioactive. The mode of action of these compounds was not known until 1991 when it was concluded that they act to inhibit complex I of mitochondrial oxidative phosphorylation with an activity several times that of rotenone (Londerhausen *et al.*, 1991). This was confirmed by Lewis *et al.*, (1993) and Ahmmadsahib *et al.*, (1993). Because of their significant bioactivity, they have attracted a lot of attention with the number of publications on the subject increasing rapidly. Uvaricin an unusual antitumour compound was the first acetogenin to be isolated and published in 1992 (Jolad *et al.*, 1982). In 1990, Rupprecht and co-workers published a review on acetogenins covering 31 compounds (Rupprecht *et al.*, 1990). This was followed by two other reviews by Fang in 1993 (Fang *et al.*, 1993; Ibid). In these two recent reviews over sixty acetogenins were covered. So far, acetogenins have only been found to be present in the genera *Annona*, *Asimina*, *Goniothalamus*, *Rollinia*, *Uvaria* and *Xylopia*. Recently, we isolated disepalin, a new acetogenin from the genus *Disepalum* (Ee *et al.*, 1996, submitted for publication).

1.3.4.1 Structural classes of acetogenins

Rupprecht (1990) in his review in 1990 placed the 31 annonaceous acetogenins into three structural subclasses of three different types of tetrahydrofuran subunits, B1, B2 and B3. Fang in this review later on introduced an additional subclass B4 with only one OH group adjacent to the THF ring (Fang *et al.*, 1993) (Figure 1.16).

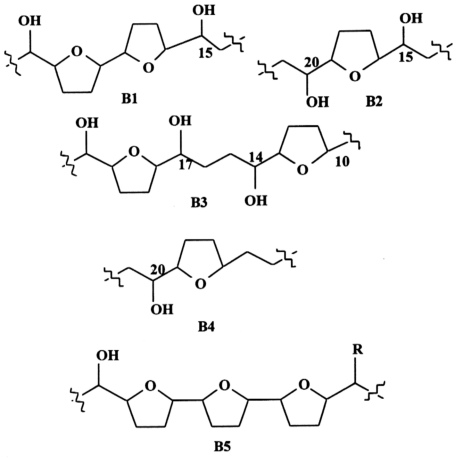


Figure 1. 16: Structures of five tetrahydrofuran subunits

Recently, type B5 acetogenins with three adjacent THF rings have been discovered (Gu *et al.*, 1994). This novel compound, goniocin was isolated from the

Goniothalamus giganteus and to date has been and still is the only acetogenin with three adjacent THF rings (refer to Figure 1.16).

These subunits are easily differentiated by their ^{13}C NMR signals. Those of the B1 type (directly linked THF rings) would have four resonances between δ 81 and δ 83; those of the B3 type (the 2 THF rings separated by a four carbon chain) have only three resonances between δ 81 and δ 83 and one at δ 79 which indicates an oxygenated tetrahydrofuran carbon lacking an adjacent hydroxyl group. The B2 types (only one THF ring) have only two ^{13}C NMR signals in the range δ 81 to δ 83. Fang , in his review in 1993, introduced a new fourth subunit which comprises four long-chain, α–β-unsaturated compounds without THF rings. Molecules containing appropriately located epoxides and/or alkenes can be construed as biogenetic percursors to the THF backbone.

The linear acetogenins all possess a characteristic γ-lactone subunit which can be differentiated by UV, IR, ^{1}H NMR and ^{13}C NMR data as well as chemical conversion. Positive reactions to Kedde's reagent and/or Legal's reagent are indicative of an α,β-unsaturated γ-lactone with compounds containing subunits A1 and A2 (Figure 1.17). Of those acetogenins containing two THF rings the γ-lactone ring can either be α,β-unsaturated as in uvaricin and anonin I or saturated with a hydroxyl group at C-33 as in itrabin. In some cases, this functionality is rearranged to an acetonylbutanolide with an oxygen at C-4 as in bullatacinone (Figure 1.18).

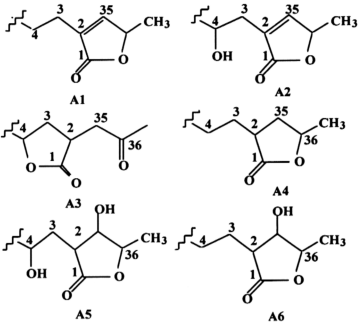
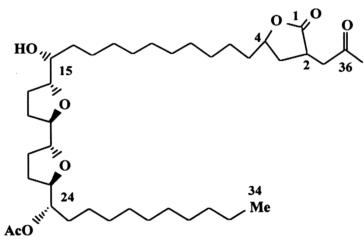
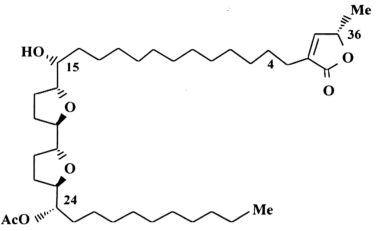


Figure 1. 17: Structures of γ-lactone units of acetogenins

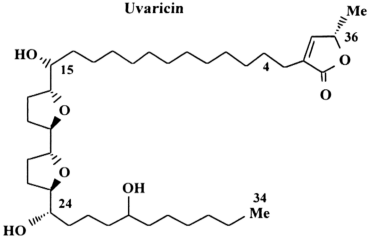


Bullatacinone

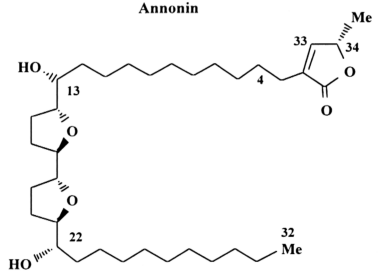
Figure 1. 18: Structure of bullatacinone



Uvaricin



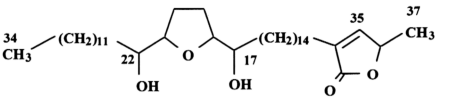
Annonin



Itrabin

Figure 1. 19: Structures of some bis-THF annonaceous acetogenins

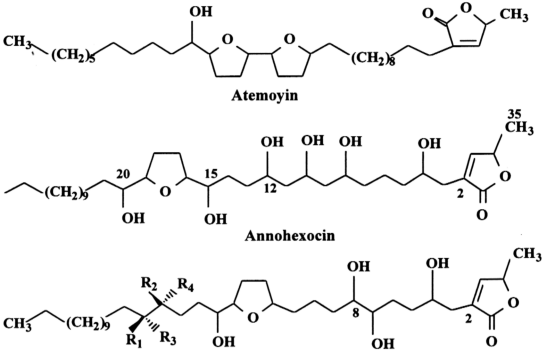
Hydroxyl groups are nearly always found adjacent to the THF rings; various other hydroxyls may be scattered along the carbon chain with the most common location at C-4, two carbons away from the lactone moiety. However, recently, Saad *et al.*, (1991) isolated reticulatacin from *Annona reliculata* which is unique among the monotetrahydrofuran ring acetogenins in that it has a different length between the lactone ring and the tetrahydrofuran ring and lacks a hydroxyl group at the 4-position (Figure 1.20).



Reticulatacin

Figure 1. 20: Structure of reticulatacin

Duret *et al.*, (1995) very recently isolated a new acetogenin belonging to the rare C_{35}-type adjacent bis-tetrahydrofuran acetogenin atemoyin from *Annona atemoya*. Again, this acetogenin does not possess a hydroxyl group at the 4-position (Figure 1.21).



	Murihexocin A	Murihexocin B
R_1	H	OH
R_2	H	OH
R_3	OH	H
R_4	OH	H

Figure 1. 21: Structures of some novel bis- and mono-THF acetogenins

More recently, McLaughlin and co-workers discovered two novel mono-THF acetogenins, annonexocin and murihexocin with six hydroxyls from *Annona muricata* (Zeng *et al.*, 1995a; Zeng *et al.*, 1995b) (Figure 1.21).

Other functional groups along the aliphatic chain are carbonyl, acetate ester, vicinal diol, epoxide and double bond moieties.

Generally, the presence of these structures is apparent from ^{1}H and ^{13}C NMR signals. The positions of the functional groups along the aliphatic chain can usually be located using the MS spectra of the acetogenins and their derivatives. Two dimensional NMR techniques helps to confirm the placement of these functional groups. The first epoxy acetogenins isolated were epoxyrollin A and B from *Rollinia ulei* (Laprevote *et al.*, 1990). Laprevote *et al.*, (1992) and Roblot *et al.*, (1992) also isolated two additional new compounds from the seeds of *Annona muricata* diepomuricanin and epomuricenin-A which contained two epoxy rings and one epoxy ring with a double bond respectively (Figure 1.22).

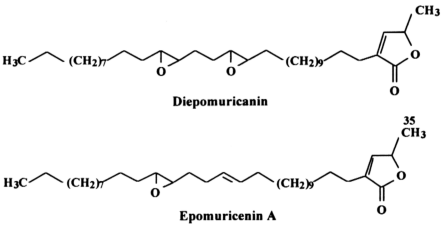
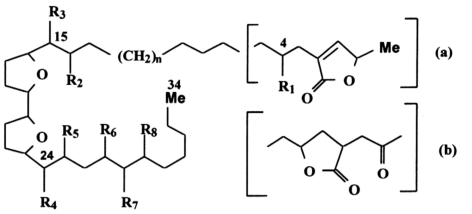


Figure 1. 22: Two novel acetogenins with epoxy rings

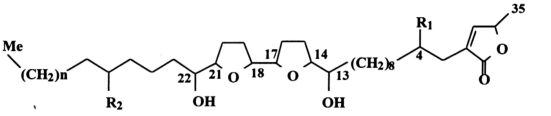
Both ^{1}H and ^{13}C NMR spectroscopy and interpretation of mass spectrometric fragmentation patterns are often used to pinpoint the location of of substituents along the carbon chains.

A. Acetogenins with adjacent bis-tetrahydrofuran ring



	n	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Type
Uvaricin	3	H	H	OH	OAc	H	H	H		
Desactyluvaricin	3	H	H	OH	OH	H	H	H		
Rollinone	3	-	H	OH	OH	H	H	H		(b)
14-Hydroxy-25-desoxyrollinicin	3	OH	H	OH	OH	H	H	H		
Asimicin	3	OH	H	OH	OH	H	H	H		
Rolliniastatin	3	OH	H	OH	OH	H	H	H		
Squamocin	3	H	H	OH	OH	H	H	OH		
Bullatacin	3	OH	H	OH	OH	H	H	H		
Bullatacinone	3	-	H	OH	OH	H	H	H		(b)
Laherradurine	3	OH	H	OH	OH	H	H	H		
Annonin I	3	H	H	OH	OH	H	H	OH		
Annonin VI	3	H	H	OH	OH	H	H	H		
4-hydroxy-25-des-oxyneorollinicin	3	OH	H	OH	OH	H	H	H		
Neoannonin	3	H	H	OH	OH	H	H	H		
Isodesacetyl-uvaricin	3	H	H	OH	OH	H	H	H		
Membranacin	3	H	H	OH	OH	H	H	H		
Trilobacin	3	OH	H	OH	OH	H	H	H		
Motrillin	3	H	H	OH	OH	H	H	H	OH	
Rioclarin	3	OH	H	OH	OH	H	H	OH	H	
Squamocin-28-one	3	H	H	OH	OH	H	H	-O-	H	

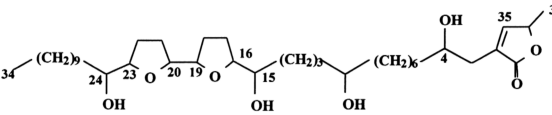
Figure 1. 23: Acetogenins with adjacent bis-THF rings



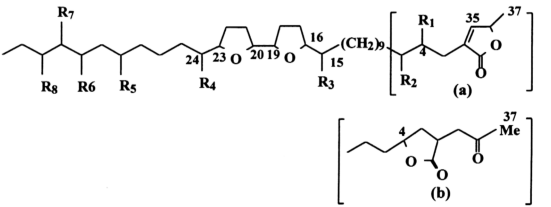
Molvizarin, R_1 = OH, R_2 = H, n = 4

Atemoyin, R_1 = H, R_2 = H, n = 8

Glaucanisin, R_1 = OH, R_2 = OH, n = 6

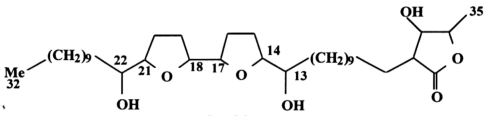


Uleicin A, B, C, D, E

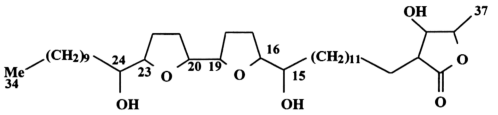


	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Type
Narumicin I	H	OH	OH	OH	H	H	H	H	
Narumicin II	H	OH	OH	OH	H	H	H	H	
Rioclarin	OH	H	OH	OH	OH	H	H	H	
Panalicin	H	OH	OH	OH	OH	H	H	H	
Squamocin-28-one	H	H	OH	OH	-O-	H	H	H	
30-OH-bullatacinone	-	H	OH	OH	H	OH	H	H	
31-OH-bullatacinone	-	H	OH	OH	H	H	OH	H	(b)
32-OH-bullatacinone	-	H	OH	OH	H	H	H	OH	

Figure 1.23: Acetogenins with adjacent bis-THF rings (continued)



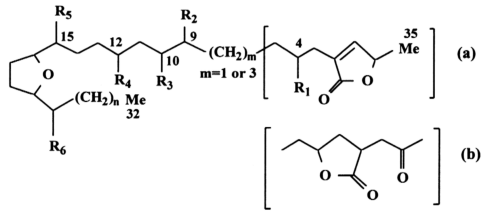
Itrabin



Laherradurin

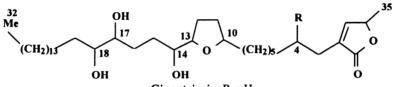
Figure 1.23: Acetogenins with adjacent bis-THF rings (continued)

B. Mono tetrahydrofuran annonaceous acetogenins



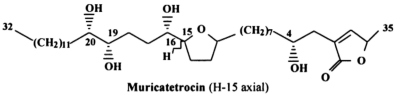
	m	R_1	R_2	R_3	R_4	R_5	R_6	n	Type
Annonacin	3	OH	H	OH	H	OH	H		
Goniothalamicin	1	OH	H	OH	H	OH	OH	13	
Squamone	3	H	-O-	H	H	OH	OH		(b)
Annonastatin	3	OH	H	H	H	OH	OH		
Annonacin-10-one	3	OH	H	-O-	H	OH	OH		
Isoannonacin	3	H	H	OH	H	OH	OH		(b)
Isoannonacin-10-one	3	H	H	-O-	H	OH	OH		(b)
Solamin	3	H	H	H	H	OH	OH		
Murisolin	3	OH	H	H	H	OH	OH		
Crossoline	3	H	H	OH	H	OH	OH		
Crossolone	3	H	H	-O-	H	OH	OH		

Figure 1. 24: Some mono-THF annonaceous acetogenins



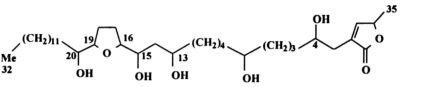
Gigantriocin, R = H

Gigantetrocin, R = OH

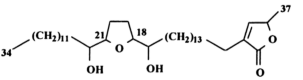


Muricatetrocin (H-15 axial)

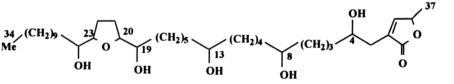
Muricatetrocin A (H-15 equatorial)



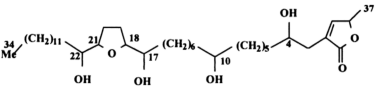
Annomonicin



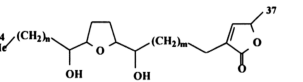
Reticulatacin



Montacin



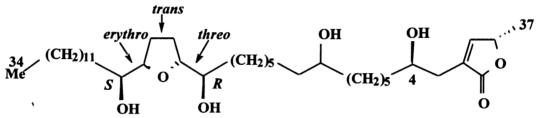
Annomontacin



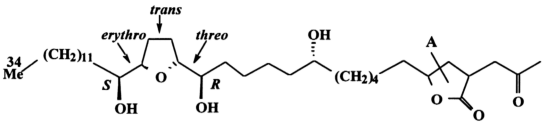
Uvariamicin I, m =11, n = 13 **Uvariamicin III**, m = 15, n = 9

Uvariamicin II, m = 13, n = 11 **Uvariamicin IV**, m = 9, n = 15

Figure 1.24: Some mono-THF annonaceous acetogenins (continued)

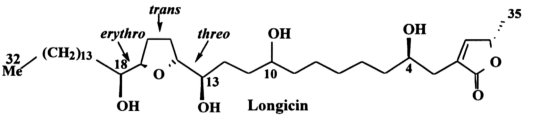


Annomutacin

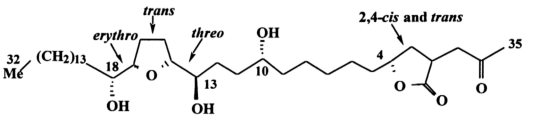


(2,4-*trans*)-10*R*-Annonacin-A-one (A = *trans*)

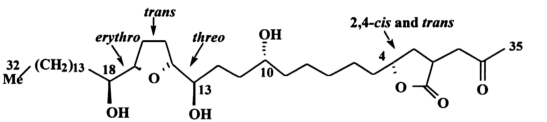
(2,4-*cis*)-10*R*-Annonacin-A-one (A = *cis*)



Longicin

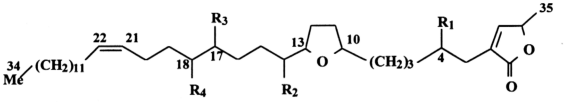


(2,4-*cis* and *trans*)-Goniothalamicinone

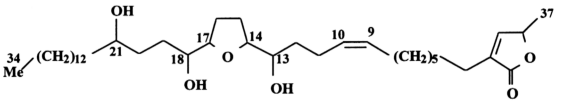


(2,4-*cis* and *trans*)-Longicinone

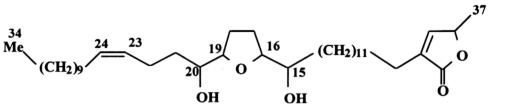
Figure 1.24: Some mono-THF annonaceous acetogenins (continued)



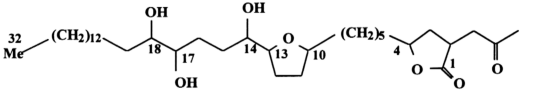
	R_1	R_2	R_3	R_4
Gigantrionenin	H	OH	OH	OH
Gigantetronenin	OH	OH	OH	OH



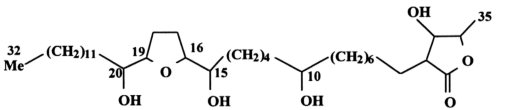
Giganenin



Bullatenin

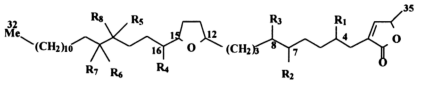


***cis*- and *trans*-Gigantetrocinone**

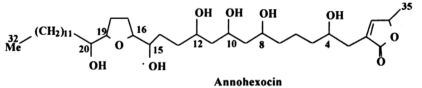


Jetein

Figure 1.24: Some mono-THF annonaceous acetogenins (continued)



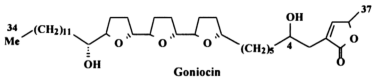
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Murihexocin A	OH	OH	OH	OH	OH	OH	H	H
Murihexocin B	OH	OH	OH	OH	H	H	OH	OH



Annohexocin

Figure 1.24: Some mono-THF annonaceous acetogenins (continued)

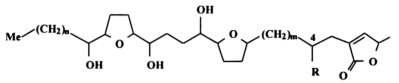
C. Tri-THF annonaceous acetogenin



Goniocin

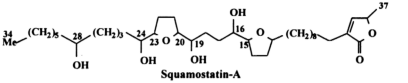
Figure 1. 25: Structure of goniocin, a tri-THF acetogenin

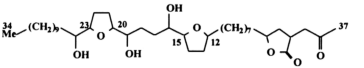
D. Non-adjacent bis-THF acetogenin



	m	n	R
Gigantecin	5	11	OH
Bullatalicin	7	9	OH
Sylvaticin	7	9	OH
4-Deoxygigantecin	5	11	H
Bullatanocin	7	9	OH

Figure 1. 26: Structures of some non-adjacent bis-THF acetogenin





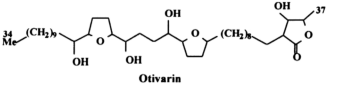
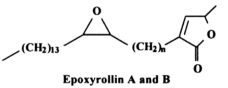
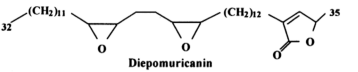
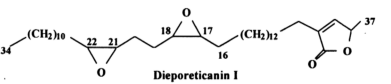


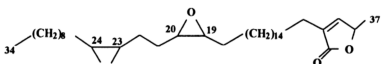
Figure 1.26: Structures of some non-adjacent bis-THF acetogenin (continued)

E. Non-THF acetogenins









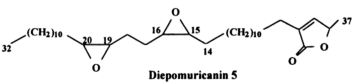


Figure 1. 27: Structures of some non-THF acetogenins

1.3.4.2 Extraction, isolation and dosage methods

Solvent extraction, solvent partition and chromatography are used, aided either by bioassay or thin layer chromatography. Fractionation, based on the lethality to brine shrimp larvae according to Meyer *et al.*, (1982) is useful because of its rapidity, its low cost and its good correlation with the antitumour activity. Thin layer chromatography can also be used. Since most of the acetogenins are characterised by the presence of an unsaturated γ-lactone, they give a coloured reaction with the Keddes reagent (2%, 3,5-dinitrobenzoic acid - 2N methanolic KOH [1:1], 5% phosphomolybdic acid in MeOH and 0.5% tetrazolium blue in MeOH-5N NaOH [1:1]). The annonaceous acetogenins typically give R_f values between 0.2 and 0.7 in the TLC plates using solvent systems $CHCl_3$-MeOH (9:1), CH_2Cl_2-MeOH (19:1), C_6H_6-EtOAC (4:6) and C_6H_6-EtOAC-MeOH (5:4:1). The Keddes reagent which gave a faint red-pink colour which fade quickly are indicative of a positive reaction indicating the presence of α,β-unsaturated γ-lactone subunit. Phosphomolybdic acid tetrazolium blue gave greyish-blue and purple chromophores, respectively. The spectrometric detection of the annonaceous acetogenins during their chromatographic separation is difficult because of their weak UV absorption.

Purification of acetogenins is rather difficult, as they often exist as a complex mixture of components of simlar polarities and they are very difficult to crystallise. Analytical HPLC of acetogenins gives good results on reverse phase silica (μ-Bondapak C18 column, mobile phase MeOH:H_2O or MeCN:H_2O, uv detection at 215 nm, flow rate 1.5 ml min^{-1}). The acetogenin of *Annona muricata*, corossolone, corossolin, murisolin and solamin were separated by HPLC using a solvent mixture of

MeCN:H_2O (85:15) as the mobile phase (Myint *et al.*, 1990; Cortes *et al.*, 1991). Londerhausen was able to separate the annonins I, III and IV and annonacin using C-18 reverse phase columns with MeOH:H_2O:methyl tert-butyl ether (MTB): propionitrile (PCN) solvent (650:300:150:2.5) (Londerhausen *et al.*, 1991). McLaughlin and his coworkers were successful in separating enantiomeric mixtures of acetogenins using normal phase 8 µm silica gel HPLC columns with gradient elution using 5.8% of a mixture of THF:methanol (1:9) in hexane (Rieser *et al.*, 1993).

1.3.4.3 Structural elucidation of acetogenins

The elucidation of acetogenin structures is very complex. Classical methods such as UV, IR, proton NMR, ^{13}C NMR and mass spectrometry are essential in identifying the structural sub-units such as methyl γ-lactone and tetrahydrofuran rings but the placement of the substituents along the carbon skeleton involves innovative mass spectrometric strategies. The location of the THF rings and oxygenated substituents in the alkyl chain requires preparation of the acetylated, silylated and the TMS derivatives and their analysis is by conventional electron impact mass spectrometry.

The diagnostic ^1H and ^{13}C NMR chemical shifts for the γ-lactone subunits are as shown in Table 1.4 (Rupprecht *et al.*, 1990).

Table 1. 4: Diagnostic ^{1}H and ^{13}C NMR chemical shifts for the γ-lactone moiety

Carbon	A1		A2		A3		A4		A5	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
C-1	-	173.7	-	174.6	-	178.3	-	175.0	-	
C-2	-	134.6	-	131.1	2.71 ddd	44.18	-	131.1	2.62-2.25	-
C-3	2.26	25.3	2.51dddd	37.4	1.70 dddd	34.41	2.40 t	25.2	2.52-2.25	-
C-4	<1.55 m	27.5	3.86 m	69.9	4.05 m	78.86	1.69 m	25.5	4.21-3.45	-
C-35	6.99 q	148.7	7.17 d	151.8	1.93 dd 2.53 dd	35.42 t	7.20 d	151.5	2.62-2.25	-
C-36	4.99 q	77.3 d	5.06 qq	78.0	-	205.44	5.06 dq	77.8	4.53 dd	-
C7	1.41 d	19.3 q	1.41 d	19.1q	1.55 s	24.46 q	1.43 d	19.1	1.36 d	-

Source: Rupprecht *et al.,* (1990).

The A2-type is easily distinguished from the Al-type by its coupling pattern in the ^{1}H NMR for the C-3 protons which are at δ 2.38 (dddd, H-3a) and δ 2.51 (ddt, H-3b) rather than at δ 2.26 (t, 2H) as well as a diagnostic ^{13}C NMR resonance for C-4 at δ 69.9. The A3 sub-unit is easily identified by its C-37 methyl ketone signal at δ 2.20(s) in the ^{1}H NMR and its C-36 carbonyl resonance in the ^{13}C NMR at δ 205.44.

The existence of THF rings with adjacent hydroxyl groups within this class of compounds is obvious by the presence of ^{13}C NMR resonances due to hydroxylated carbons in the region from δ 71 to δ 75 and other oxygenated carbons in the region δ 79-84 corresponding to the THF oxygenated carbons. These signals and their corresponding ^{1}H NMR resonances from δ 3.3 to δ 4.1 indicate the dihydroxyl THF moieties.

1.3.4.4 Stereochemistry

There are usually 5-10 chiral carbons in the structure of the annonaceous acetogenins. As mentioned earlier, the waxy nature of these compounds does not

allow for direct X-ray crystallographic studies, hence the stereochemistry of most acetogenins have not been completely determined. The region of greatest stereochemical variation is the THF-containing moieties (up to six chiral centres). Most recently published acetogenins have their relative configurations at the THF moieties determined by comparing the ^{1}H NMR resonances of their acetates with those of a group of synthetic diastereoisomeric dibutyl diacetylated bis THF models of known relative stereochemistry. Careful comparison of the ^{1}H NMR spectra of these compounds indicated that stereochemical information could be obtained from careful analysis of the very small differences in their high-field NMR chemical shifts (Hoye and Suhadolnik, 1987; Hoye and Zhuang, 1988). The application of this technique was first successfully applied to the acetogenins uvaricin, asimicin and rolliniastatin (Hoye and Suhadolnik, 1987; Hoye and Zhuang, 1988).

A second technique for determining stereochemistries of the THF portion of acetogenins has been described by Born *et al.*, (1990). In this method, minor differences in the ^{1}H and ^{13}C NMR chemical shifts of two models (one with *threo* and the other with *erythro*) are compared with the corresponding signals of the natural product acetogenin annonin. Stereochemical information can be abstracted from the comparison data.

For further validation of Hoye's and Born's methods, the diagnostic NMR chemical shifts corresponding to different THF subunits with various relative configuration in acetogenins and their acetates have been summarised (Table 1.5) (Fang *et al.*, 1993). The obvious correlations between the NMR data and the various relative configurations of these acetogenins and their acetates support the applicability

of these two methods for the stereochemically different THF subunits in the different acetogenin subclasses.

Table 1: 5: Diagnostic NMR chemical shifts of different subunits with various relative configuration in acetogenins

Compound	^{1}H (^{1}H of Acetate)						^{13}C					
	1	2	5	6	9	10	1	2	5	6	9	10
Asimicin	3.37	3.84	3.84	3.84	3.84	3.37	74.0	83.1	81.8	81.8	83.1	74.0
	4.85*	3.98*	3.90*	3.90*	3.98*	4.85*						
Isodesacetyl uvaricin	3.39	3.85	3.85	3.85	3.85	3.39	74.1	83.2	81.8	81.8	83.2	74.1
	4.85*	3.98*	3.89*	3.89*	3.98*	4.85*						
Bullatacin	3.83	3.92	3.83	3.92	3.83	3.38	71.3	82.8	82.2	82.4	83.2	74.1
	4.93*	4.00*	3.90*	3.90*	4.00*	4.88*						
Squamocin	3.90	3.90	3.90	3.90	3.90	3.38	71.3	82.6	82.1	82.4	83.2	74.0
	4.93*	3.98*	3.89*	3.89*	3.98*	4.85*						
Squamocin-28-one	3.90	3.90	3.90	3.90	3.90	3.38	71.3	82.7	82.1	82.4	83.2	74.0
	4.93*	3.98*	3.89*	3.89*	3.98*	4.85*						
Trilobacin	3.34	3.80	3.93	4.01	3.80	3.34	74.6	83.3	81.6	80.9	82.6	73.9
	4.85*	3.96*	3.82*	3.82*	3.96*	4.85*						
Rolliniastatin 1	3.85	3.85	3.85	3.85	3.85	3.38	71.8	83.0	81.0	81.1	83.0	74.0
4-Hydroxy-25 desoxyneo-rollinicin	3.84	3.84	3.84	3.84	3.84	3.41	71.9	82.9	81.0	81.1	83.0	74.0
	4.91*	3.95*	3.86*	3.86*	3.95*	4.91*						
Annonacin -A	3.82	3.82	3.82	3.40			71.6	83.3	82.3	74.4		
	4.91*	3.98*	3.98*	4.83*								
Aonnacin-A-one	3.85	3.79	3.79	3.38			71.7	82.2	82.2	74.3		
	4.89*	3.94*	3.94*	4.81*								
Bullatalicin	3.87	3.87	3.80	3.41			71.5	83.3	82.0	74.4		
	4.91*	3.97*	3.97*	4.82*								
Bullatalicinone	3.87	3.87	3.81	3.41			71.4	83.3	82.2	74.4		
	4.91*	3.96*	3.96*	4.83*								
Annonastatin	3.40	3.84	3.84	3.40			74.1	83.2	81.8	74.1		
	4.85*	3.97*	3.97*	4.85*								
Bullatenin	3.42	3.81	3.81	3.42			74.1	82.7	82.7	74.1		
	4.90*	3.97*	3.97*	4.87*								
Bullatanocin	3.41	3.80	3.80	3.41			74.0	82.7	82.7	74.2		
	4.83*	3.96*	3.96*	4.83*								
Giganenin	3.41	3.80	3.80	3.41			73.5	82.6	82.6	74.3		
	4.86*	3.98*	3.98*	4.86*								
Gigantriocin	3.43	3.81	3.88				74.4	81.8	79.3			
	4.79*	3.93*	3.83*									
Gigantetrocin	3.40	3.80	3.87				74.4	81.8	79.3			
	4.78*	3.92*	3.82*									
Bullatalicin	3.41	3.80	3.87				74.4	82.0	79.2			
	4.82*	3.97*	3.86*									
Bullatanocin	3.41	3.80	3.87				74.2	82.0	79.2			
	4.83*	3.96*	3.85*									

* Values for ^{1}H of Acetate, Source: Fang *et al.*, (1993).

Assignments done by using the Hoye's and Born's method are in agreement with the assignments determined by X-ray crystallographic data for 15-O-*p*-

bromophenylurethane derivative of rolliniastatin 1 and for a derivative of squamocin (annonin 1).

However useful the methods described by Hoye and Born were, the question of absolute confirugation was still left open. In 1992, Hoye collaborated with the McLaughlin group to determine the absolute stereochemistry of the carbinol centres of nine natural acetogenins using the Mosher ester (MTPA or methoxytrifluoromethylphenylacetate) method (Rieser *et al.*, 1992). These acetogenins include five adjacent bis-THF acetogenins and four mono-tetrahydrofuran acetogenins. In the original method described by Mosher, the absolute stereochemistry of an unknown stereocentre could be determined by observing the shielding of proton adjacent to the carbinol after it is esterified with MTPA. The two enantiomers of the Mosher acid chloride *(R)*-MTPA-Cl and *(S)*-MTPA-Cl are used to derivatise a stereogenic carbinol center to the *(S)*- and *(R)*-MTPA esters, respectively.

Since all THF-containing acetogenins have at least one hydroxyl group in the B subunit, Mosher derivatisation of these groups produces an opportunity to draw conclusions about the absolute configuration in this portion of the molecule. Owing to the diamagnetic effect of the benzene ring the $H_{a,b,c}$ NMR signals of the *(R)*-MTPA ester should appear upfield relative to those of the *(S)*-MTPA ester. The reverse should hold true for $H_{x,y,z}$. Therefore, when $\Delta\delta = \delta_S - \delta_R$, protons on the right side of the MTPA plane must have positive values ($\Delta\delta>0$) and protons on the left side of the plane must have negative values ($\Delta\delta<0$) as illustrated (Fang *et al.*, 1993).

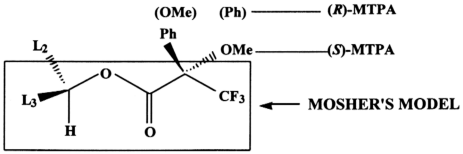


Figure 1. 28: Mosher's model for the (*R*)-MTPA and (*S*)-MTPA derivatives

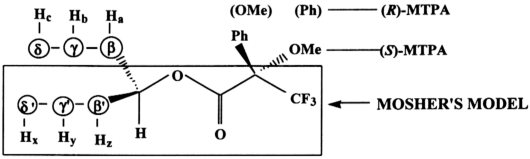


Figure 1. 29: The MTPA plane of an MTPA ester and $H_{a,b,c}$ and $H_{x,y,z}$ on the right and left sides of the plane, respectively

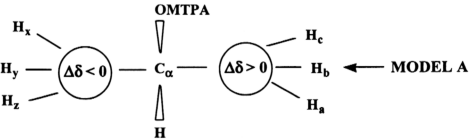


Figure 1. 30: Model A for the determination of the absolute configuration of secondary alcohols (Ohtani *et al.*,1991)

Hence, Mosher's method is:-

1. Prepare the (*R*)- and (*S*)-MTPA esters and assign as many proton resonances as possible

2. Obtain $\Delta\delta$ (δ_S - δ_R) values for the protons

3. Place those protons with a (-) $\Delta\delta$ on the left of the MTPA plane and those with a (+) $\Delta\delta$ on the right side of the plane

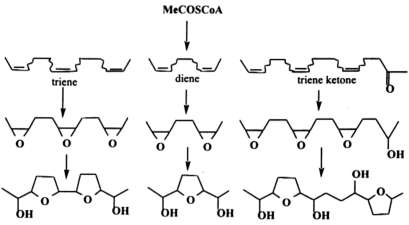
4. Validate the stereochemical assignments by confirming that all protons with (+) and (-) actually reside on the expected side of the plane.

The absolute stereochemistry about the carbinol position C-4 of all natural 4-hydroxylated acetogenin that have so far been examined have been found to be *R*. However, the ketolactone compounds are assumed to retain the *R* configuration (Fang *et al.*, 1993).

1.3.4.5 Biosynthesis

No experimental work on the biosynthesis of acetogenins have been reported since they are a relatively new class of compounds. Several proposals have been made regarding the natural origin of the adjacent bis-tetrahydrofuran rings acetogenins. Since the acetogenins are long chain compounds with a γ-lactone moiety and one or more tetrahydrofuran rings incorporated in the chain it is clear that they are derived from the polyketide pathway. The precursors could have been assembled by the linear combination of two and three carbon units (acetic acid and propanoic acid) via acetyl-CoA, malonyl-CoA and propanyl-CoA, through mechanisms analogous to the well-known pathway for fatty acid biosynthesis. Based on this prediction, the different classes of the THF rings can be obtained by epoxidation of triene, diene or triene-ketone intermediates followed by ring openings and closures (Rupprecht *et al.*, 1990) (Figure 1.31). Stereocentres due to the THF rings and adjacent hydroxyl

groups arise from the regiochemistry of double bonds, the stereospecificity of epoxidation and the opening of the epoxide rings.



Triene: uvaricin,rolliniastatin, annonin 1, annonin VI, 4-hydroxy-25-deoxy-neorollinicin, asimicin, bullatacin andbullatacinone

Diene: annonacin, goniothalamicin, squamone, annonastatin annonacin A

Triene ketone: gigantecin,bullatalicin, sylvaticin

Figure 1. 31: Hypothesis for the biosynthesis of the THF rings of annonaceous acetogenins.

The lactone ring is presumed to be formed by an aldol-type condensation involving a three carbon compound. This is of a type long known to be used in biosynthesis of fatty acid derivatives.

Jolad *et al.*, (1982) proposed that uvaricin is probably biosynthesised from tetratraconta-15,19,23-trienoic acid via triepoxidation followed by addition of HOAc.

A series of acetogenins such as gigantetronenin and gigantrionenin isolated from the *Goniothalamus giganteus* strongly supports a polyketide pathway as hypothesised by Fang (Fang *et al.*, 1992; Fang *et al.*, 1993) (Figures 1.32 and 1.33).

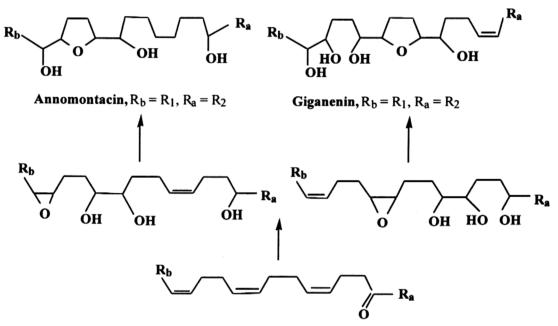


Figure 1. 32: Biogenetic pathways leading to the acetogenins from *G. giganteus*.

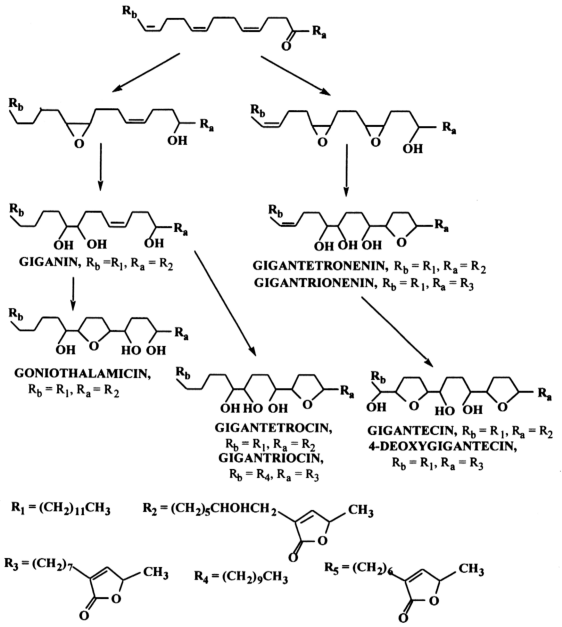


Figure 1. 33: Biogenetic pathways leading to the acetogenins from *G. giganteus*

Some epoxy-containing acetogenins isolated from the leaves of *Annona muricata* (Laprevote *et al.*, 1990; Laprevote *et al.*, 1992a) gave further convincing evidence for the polyketide biogentic pathway of acetogenins (Figure 1.34).

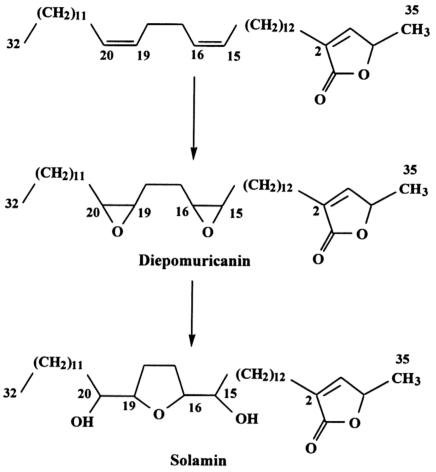


Figure 1. 34: A possible role of diepomuricanin in the biogenesis of solamin (Laprevote *et al.*, 1992)

1.3.4.6 Biological activity of annonaceous acetogenins

Annonaceous plant extracts have been used widely as pesticides, antitumour agents and so on. Undoubtedly, many of these previously unexplained folkloric uses can be attributed to this new class of compounds, the acetogenins which are very bioactive.

1.3.4.6.1 Cytotoxicity activity and antitumour

Many plant members in this family have been used in folk medicine as anticancer agents (Rios *et al.*, 1989; Pettit *et al.*, 1987; Fujimoto *et al.*,1988).

A. Mechanism of action. Londerhausen *et al.*, (1991) showed that the acetogenins are inhibitors of NADH ubiquinone reductase (complex 1) of oxidative phosphorylation in the mitochondria. They observed that the toxicity exhibited by insects exposed to annonaceous acetogenins included a slow decrease in mobility and an increase in lethargy before death. Such symptons are usually due to a lowering of ATP levels caused by respiratory inhibitors. Annonin 1 (squamocin), anthimycin A (an ATP synthase inhibitor), cyfluthrin and parathion (two neurotoxic compounds) and an untreated control were all tested for their ability to reduce ATP levels in fourth instars of *Plutella xylostella* (Londerhausen *et al.*, 1991). The ATP levels of those animals treated with annonin I and antimycin A were 30% lower than the control or those treated with neurotoxins. After determining that respiration had been affected, a series of experiments using mitochondria and well-characterised respiratory inhibitors (cyanide, rotenone and antimycin A) were carried out to determine the site-specific effect on the mitochondrial electron transport complexes. Photometric determinations of the redox state of the various cytochromes indicated that the arrest of electron flow occurred at NADH-ubiquinone reductase (complex 1). This result was reproducible with oxygen-electrode polarographic experiments.

The inhibition of oxygen consumption was reversed by the addition of α-glycerophosphate; the addition of an artificial electron carrier (N,N,N,N'-tetramethyl-*p*-phenylenediamine dihydrochloride, TMPD) restored the flow of electron and the

initial addition of oligomycin and dinitrophenol (DNP) (uncoupling agent) had no effect on the inhibition exhibited by annonin I. This is evidence that the primary mode of inhibition is arrest of electrons transport at complex I. The inhibition was 2-4 times more effective than that exhibited by rotenone. The Fe-S centres of complex I were reduced by NADH in mitochondria inhibited by annonin I. This indicated that electron flow is arrested just before the reduction of ubiquinone.

Lewis *et al.*, (1993) performed *in vivo* experiments using extracts from *Asimina triloba* and the European corn borer (*Ostrinia nubilalis*). The group also carried out *in vivo* experiments using pure asimicin with insect mitochondrial preparation. They arrived at the same conclusions as Londerhausen *et al.*, (1991).

B. Structure-activity relationships. The cytotoxicity data tabulated in Table 1.6 illustrates a number of structure-activity relationships among the acetogenins. From the data it is gathered that the most potent cytototoxic compounds possess an adjacent bis-THF ring sub-unit; the non-adjacent bis-THF ring acetogenins is less cytotoxic while the mono-THF compounds is the least cytotoxic. The γ-lactone ring is necessary for activity. α,β-unsaturated γ-lactone are more active than the saturated lactone. Ketolactones are less potent than α,β-unsaturated γ-lactones; reduction of the ketone restores some activity. The greater the activity of the compound, the greater the extent of hydroxylation. Also, the position of the hydroxyl groups is critical. Acetylation of the hydroxyl groups of rollinicin, bullatacin, bulaltacinone, bullatalicin resulted in a decrease in cytotoxicity. Furthermore, reduction of the ketone moieties of squamone resulted in a substantially increased level of cytotoxicity. The hydroxyl group at the C-4 position is very important for the enhancement of activity; distant

hydroxyls for instance at C-28 to C-32 also show enhancement. This suggests that oxidation of the hydroxyl groups to ketones would decrease activity while reduction of the natural keto groups to hydroxyls could increase activity. The presence of vicinal diols, acetate derivatives and probably other susbtitutions on the hydroxyls produce compounds which are less active than their parent compounds. Uvaricin, the first annonaceous acetogenin to be isolated was claimed as a new antitumour agent (Jolad *et al.*, 1982). However, it presents a rather important *in vivo* cytotoxic activity against lymphocytic leukemia 3PS with a T/C of 160% at the dose of 1.4 mg/kg.

It is now evident that all the acetogenins possess, to a varying degree, cytotoxic acitivty. Against KB cells, this toxicity appears at a concentration between 10^{-1} and 10^{-5} mg/ml depending on the nature of the functional groups and the stereochemistry. Acetogenins isolated from annonaceous plants that have shown significant cytotoxicity and antitumour *in vivo* activity are listed in Table 1.6 (Rupprecht *et al.*, 1990).

Table 1. 6: Cytotoxicity and antitumour activity of annonaceous acetogenins

	In vitro							*In vivo*	
Compound	**9PS**	**9KB**	**A549**	**MCF-7**	**HT-29**	**9ASK**	**L1210**	**3PS**	**L1210**
	ED_{30}(µg/ml)							%T/C(mg/kg)	
Uvaricin	-	-	-	-	-	-	-	157% (1.4)	-
Rolinicin	2.9×10^{-8}	-	-	-	-	-	-	-	-
Rolinicin triacetate	2.6×10^{-4}	-	-	-	-	-	-	-	-
Isorollinicin	10^{-2}	-	-	-	-	-	-	-	-
Rollinone	$<10^{-5}$	-	-	-	-	-	-	147% (1.4)	-
Cherimoline	$<10^{-2}$	6×10^{-12}	10^{-3}	-	$>10^{-3}$	inactive	-	-	-
Dihydrocherimoline	$<10^{-2}$	2×10^{-8}	10^{-3}	-	10^{-3}	inactive	-	-	-
Asimicin	$<10^{-12}$	$<10^{-5}$	10^{-3}	-	3.3×10^{-11}	inactive	-	124% (0.025)	131% (0.2)
Roliniastatin 1	4.5×10^{-5}	-	-	-	-	-	-	128% (0.25)	-
Rolliniastatin 2	2.3×10^{-4}	-	-	-	-	-	-	-	-
Squamocin	-	-	-	-	-	-	0.58	-	-
Bullacacin	10^{-13}	6.2×10^{-14}	1.3×10^{-13}	<10	10^{-12}	inactive	-	-	138% (0.05)
Bullacacin triacetate	3.9×10^{-3}	6.9×10^{-7}	2×10^{-3}	-	$<10^{-1}$	-	-	-	-
Dihydrobullacacin	-	-	$<10^{-6}$	-	3.3×10^{-3}	-	-	-	-
Bullacacinone	4.2×10^{-3}	$<10^{12}$	10^{-3}	-	5×10^{-12}	inactive	-	-	144% (0.4)
Bullacacinone diacetate	4.2×10^{-2}	5×10^{-3}	2.8×10^{-2}	-	10^{-1}	-	-	-	-
4-Hydroxy-25-desoxyneo rollinicin	-	-	$<10^{-3}$	$<10^{-3}$	1.26	-	-	-	-
Annonacin	10^{-3}	10^{-3}	10^{-3}	-	3	51% 15-30%	-	124% (0.95)	-
Goniothalamicin	$<10^{-1}$	$<10^{-2}$	-	-	-	-	-	-	-
Squamone	-	-	1.34	2.14	1.5	-	-	-	-
Tetrahydrosquamone	-	-	1.4×10^{-1}	9.9×10^{-4}	3.0×10^{-1}	-	-	-	-
Annonacin-10-one	10^{-6}	-	10^{-1}	-	1	-	-	-	-
Isoannonacin	3	-	2×10^{-2}	-	2×10^{-4}	-	-	-	-
Isoannonacin-10-one	5×10^{-1}	-	7×10^{-2}	-	9×10^{-4}	-	-	-	-
Gigantecin	$<10^{-2}$	$<10^{-3}$	2.2×10^{-7}	4.1×10^{-9}	2.9×10^{-4}	31-50%	-	-	-
Bullatalicin	-	>10	2.3×10^{-7}	2.34	8.8×10^{-6}	-	-	-	-
Bullatalicin tetraacetate	-	-	$<10^{-1}$	$<10^{-1}$	1.35	-	-	-	-
Sylvaticin	-	$<10^{-4}$	$<10^{-4}$	-	$<10^{-4}$	-	-	-	-

Source: Rupprecht *et al.*, (1990).

In 1990, McLaughlin and co-workers submitted a number of acetogenins for evaluation of cytotoxic and antitumour activity (NIH/NCI human tumour cell panel). Table 1.6 shows the results of these tests (Rupprecht *et al.*, 1990). The test results indicated no major differences between the compounds tested and most of the acetogenins gave a similar spectrum of activities in the NCI Compare program. All

the compounds showed similar GI_{50} values ranging from 10^{-5} to 10^{-7} ug/ml in the three-day NCI test. (GI_{50} is the concentration of compound needed for 50% growth inhibition as compared to the control). However, with longer run-times (7-day runs) the bis-THF acetogenins often show potencies with ED_{50} values below 10^{-12} ug/ml. They speculated that substrate-level phosphorylation might play a greater role in bioenergetics in shorter run period while longer runs exhaust anaerobic sources of ATP production and rely more heavily on oxidative phosphorylation by the mitochondrial electron transport system.

In vivo antitumour activity evaluation have been carried out on several acetogenins at the Upjohn Company (Ahmmadsahib *et al.*, 1993). Table 1.7 shows the results for L1210 murine leukaemia. There is a significant activity for bullatacin and bullatacinone; similar data for taxol were taken from the NCI file for comparison. From this comparison, it is seen that bullatacin and bullatacinone possess respectively 300 times and 40 times the potency of taxol and similar positive antileukaemic effects.

Table 1. 7: *In vivo* antitumour activities of bullatacin, bullatacinone, bullatalicinone and squamocin against i.p.-implanted L1210 leukaemia

Compound	Daily dose (mg/mL)	Survival 1st run (% T/C)	Survival 2nd run (% T/C)	Weight changes (g/mouse)
Bullatacin	200	toxic	toxic	-2.9
	100	106	100	-2.6
	50	138	138	-0.4
	25	125	131	0.6
	12.5	113	138	0.9
Bullatacinone	800	144	-	0.5
	400	144	-	1.1
	200	125	-	1.8
	100	113	-	1.6
	50	113	-	2.1
	25	100	-	1.7
Bullatalicinone	5000	toxic	-	-0.9
	2500	100	-	-1.1
	1250	113	-	-0.5
	625	113	-	0.3
Squamocin	5000	toxic	-	-
	2500	toxic	-	-
	1250	106	-	-1.4
	625	113	-	-1.1
Taxol	25000	90	-	-4.4
	17500	105	-	-2.4
	15000	139	-	-2.5
	7000	114	-	-1.1
	4400	117	-	-0.9

Source: Ahammadsahib *et al.*, (1993).

Earlier cytotoxicity results for bullatacin and asimicin in seven-day tests at NCI showed potent selectivity against A2780 human ovarian tumour cells (Hui *et al.*, 1989). Bullatacin and bullatalicin showed significant 10-day tumour growth

inhibition at respective doses of 0.05 and 1.0 mg/kg/day with much less weight loss than cisplatin.

Table 1. 8: Antitumour efficacy of bullatacin, bullatacinone and bullatalicin in athymic mice bearing s.c.-implanted A2780 human ovarian carcinoma xenografts

Compound	Dose (mg/kg/day)	Day 10% TGI	Weight Change (g/mouse)	Death Total
Bullatacin	0.10	68	-1.5	2/8
	0.050	67	-0.3	0/8
	0.025	35	+0.1	0/8
Bullatacinone	0.5	33	-0.7	1/8
	0.25	27	0.1	0/8
	0.125	52	-0.4	0/8
Bullatalicin	2.0	63	-0.9	2/8
	1.0	75	-0.1	0/8
	0.5	42	-0.2	0/8
Cisplatin[a]	5.0	78	-1.1	0/8
ventricle control	-	-	-0.3	0/8

[a] Cisplatin was concurrently tested for comparison and was given on day one only as a single dose.
Source: Ahammadsahib *et al.*, (1993).

Bullatacin and bullatacinone are being patented in the United States as chemotherapeutic agents (McLaughlin and Hi, 1993). A similar Japanese patent has been granted for squamostatin A and its analogues (Ikekawa *et al.*,1991).

1.3.4.6.2 Pesticidal activity of annonaceous acetogenins

It is notable that in some countries of South America ground barks or seeds of some species of Annonaceae are spread on soils as pesticides. In Malaysia, plants such as *Goniothalamus* species, are well known to be insecticidal and the bark is used

as an insect repellent. Moeschler *et al.*, patented annonin, the structure for which was still unknown and not defined as an acetogenin as an insecticidal compound in 1984 (Moeschler *et al.*, 1984; Moeschler *et al.*, 1987). In 1988, Mikolajczak *et al.*, patented the entire group of annonaceous acetogenins as pesticides (Mikolajczak *et al.*, 1988). In this patent, asimicin was claimed as an example for the first structurally defined pesticidal acetogenin. Recent research at Agridyne (Mikolajczak, unpublished results) had demonstrated that the acetogenin-containing extracts of the bark of *Asimina triloba* (paw paw) and the seeds of *Annona muricata* (sour sop or guanabana) are potent as pesticides against aphids, white flies and Colorado potato beetles at concentrations of 300-5000 ppm. The seeds of *Annona glabra* gave asimicin, squamocin and desacetyluvaricin which were not only lethal but also showed feeding deterrent and growth inhibitory effects in a variety of insects (Ohsawa *et al.*, 1991). Other acetogenins have subsequently been reported to have pesticidal activity such as bullatacin (Hui *et al.*, 1989), annonacin (McCloud *et al.*, 1988), goniothalamicin (Alkofahi *et al.*, 1988) and sylvaticin (Mikolajczak *et al.*, 1990). Synergisms between the acetogenins containing extracts of *Annona glabra* and pyrethrins as well as azadiractin-containing neem seed extracts show the commercial promise of combination products with the acetogenin as new, natural, environmentally compatible pesticides. Annonin (squamocin) and neoannonin showed strong ovicidal and larvicidal activity in the *Drosophila* feeding test at 125-140 µg/2g of diet for *Drosophila melanogaster* (Kawazu *et al.*, 1989). Evaluation of a standardized crude extract of the bark of *Asimina triloba* shows promise as a garden pesticide (Poncavage, 1989).

Table 1. 9: Pesticidal activity of tested acetogenins

Compound	Concentration µg/ml	MBB	MA	ML	NE	BFL	SCB	SAW	CRW	TSSM
		% Mortality								
Asimicin	5000	-	-	-	-	-	50	-	-	-
	1000	-	-	-	-	100	-	-	-	-
	500	100	100	-	-	-	-	-	-	-
	100	100	20	100	100	0	-	-	-	-
	50	100	0	-	-	-	-	-	-	-
	10	70	0	100	100	0	-		-	-
	1	-	-	100	100	0	-	-	-	-
	0.1	-	-	75	1	-	-	-	-	-
Bullatacin	400	-	90	-	-	-	-	0	-	20
	100	-	80	-	-	-	-	0	-	30
	24	-	-	-	-	-	-	-	80	-
	10	-	80	80	-	-	-	0	-	20
	6	-		-	-	-	-	-	20	-
	1	-	80	0	-	-	-	-	-	-
	0.5	-	-	0	-	-	-	-	-	-
Annonacin	10000	-	-	-	-	100	-	-	-	-
	10	-	-	70	-	-	-	-	-	-
Goniothalamicin	10000	-	-	-	-	100	-	-	-	-
Sylvaticin	-	-	-	-	-	-	active	-	-	-
Annonin VI	-	-	-	-	active	-	-	-	-	-

Source: Rupprecht *et al.*, (1990).

1.3.5 Annonaceous acetogenins from the genus *Goniothalamus*

The first acetogenins to be isolated from the genus *Goniothalamus* were goniothalamicin and annonacin. These two mono-THF acetogenins were found in *Goniothalamus giganteus* (Annonaceae) by Alkofahi and co-workers (Alkofahi *et al.*, 1988). Two years later, the same group isolated gigantecin, a novel non-adjacent bis-THF acetogenin from the same plant (Alkofahi *et al.*, 1990). More recently, Fang *et al.*, isolated two mono-THF acetogenins with a double bond at C-21 from *G. giganteus* (Fang *et al.*, 1992). Gu and co-workers have isolated the first tri-THF acetogenin, goniocin from *G. giganteus* (Gu *et al.*, 1994). Recently, we too isolated the acetogenin annonacin from *Goniothalamus dolichocarpus* (Goh *et al.*, 1995a); *G. malayanus*, *G. velutinus* and *Mezzetia umbellata* (Ee, G.C.L.,unpublished data). We also isolated a new mono-THF acetogenin with an acetoxy group adjacent to the THF

ring from *Disepalum anomalum*. For the first time, the genus *Disepalum* has provided a mono-THF acetogenin which has an acetoxy group adjacent to the THF ring (Ee *et al.*, 1996 submitted for publication).

1.3.6 Alkaloids from the genus *Goniothalamus*

The first report on the isolation of alkaloids from the *Goniothalamus* species was in 1985 when Lu *et al.*, isolated three alkaloids from *Goniothalamus amuyon*. These alkaloids were the aporphines, (-)-anolobine and (-)-anonaine together with the oxoaporphine liriodenine (Lu *et al.*,1985) (Figure 1.35). Three years later, Talapatra and co-workers published their findings whereby they isolated from *Goniothalamus sesquipedalis* three alkaloids, goniopedaline, aristolactam A-II and taliscanine (Talapatra *et al.*,1988) (Figure 1.36). These are all phenanthrene lactams. This was the first report of the phenanthrene lactams from the *Goniothalamus* genus. We isolated the phenanthrene lactam aristolactam B-II and the dioxoaporphine ouregidione from *G. malayanus* and *G. velutinus* (Ee, G.C.L., unpublished data).

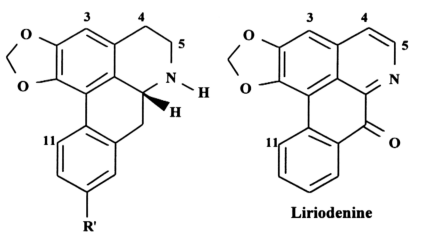
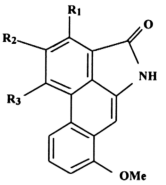


Figure 1. 35: Structures of (-)- anolobine, (-)-anonaine and liriodenine



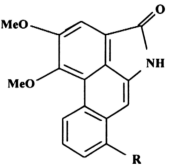
Goniopedaline, R_1 = OMe, R_2 = OH, R_3 = OMe

Aristolactam AII, R_1 = H, R_2 = OH, R_3 = OMe

Taliscanine, R_1 = H, R_2 = OMe, R_3 = OMe

Figure 1. 36: Structures of goniopedaline, aristolactam AII and taliscanine

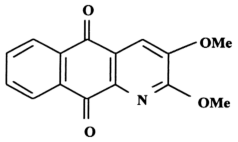
The third report on the presence of phenanthrene lactam in *Goniothalamus* species was by Omar and co-workers whereby they isolated a new phenanthrene lactam velutinam from *Goniothalamus velutinus* (Omar *et al.*, 1992). Along with this new alkaloid, was also isolated Aristolactam BII (Figure 1.37).



Velutinam, R = OH
Aristolactam BII, R = H

Figure 1. 37: Structures of velutinam and aristolactam BII

Goniothalamus scortechinii gave the anthraquinone scorazanone which is a 1-aza-anthraquinone (Din *et al.*, 1990) (Figure 1.38).



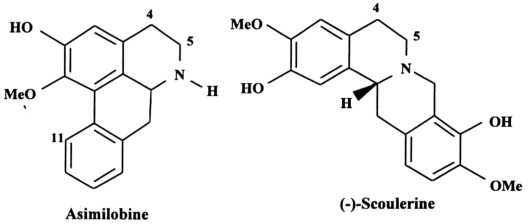
Scorazanone

Figure 1. 38: Structure of scorazanone

In this work, the phenanthrene lactam Aristolactam BII was isolated from the *Goniothalamus velutinus* and *Goniothalamus malayanus* while the *G. velutinus* also gave an oxoaporphine ouregidione.

1.3.7 Compounds isolated from the genera *Disepalum* and *Mezzetia*

No species of *Disepalum* has so far been thoroughly investigated. Lavault and co-workers have studied the *Disepalum pulchrum* from Malaysia and isolated five known isoquinoline alkaloids namely (-)-norliridine, liriodenine and (-)-scoulerine (Lavault *et al.*, 1990) (Figure 1.39). No new compounds were isolated from this species. We have studied the species *Disepalum anomalum* and isolated from it the known oxoaporphine liriodenine together with a new acetogenin.



Asimilobine **(-)-Scoulerine**

Figure 1. 39: Structures of asimilobine and (-)-scoulerine

The genus *Mezzetia* is equally as poorly investigated as the genus *Disepalum*. Only one species has been studied that is, *Mezzetia leptopoda*. Powell and co-workers isolated mezzetiaside 3, a highly acylated trisaccharide (Powell *et al.*,1990). We have isolated the acetogenin annonacin and an inseparable amount of goniothalamicin from *Mezzetia umbellata*.