CHAPTER I

INTRODUCTION

The mangrove forest or sometimes called tidal forest covers the areas where sea water can flood. Mangroves are widespread in the tropical and subtropical regions of the world. The mangrove area is usually muddy and full of useful organic substances as well as mineral elements for living organisms in this environment. Therefore, the mangrove areas are important as natural resources in cases of forestry, fisheries and the preservation of the ecosystem.

In the present day, the total mangrove area in the world is approximately 15.48 million hectar distributing among many tropical countries, while the mangrove area in Thailand is only about 0.27 million hectar distributing in the southern and southeastern parts of the country and the upper part of the Gulf of Thailand.[1] There are more than 60 species (approximately 27 genera and 13 families) found in Thai mangrove areas. The community structure of Thai mangrove areas is varying from the edge of the estuary to inland sites, with *Rhizophora apiculata*, *R. mucronata* and the plam, *Nypa fruticans*, the dominant species along estuary and chemical edge. *Avicennia* and *Bruguiera* associated with *Rhizophora* formed a more distinct zone further inland. On areas adjacent to the

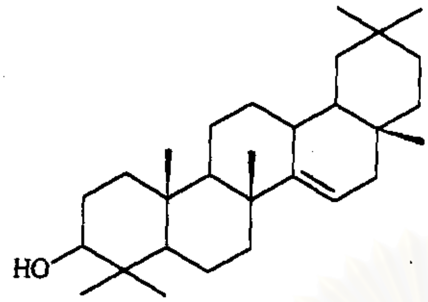
Avicennia and *Bruguiera* zone which have drier soils and loss to tidal in undation, *Xylocarpus* and *Excoecaria* become the dominant species.[2]

Mangrove forests are known to be coastline stabilizers by retaining and building of the land, reserviors for wastes assimilation, the global cycle of carbon dioxide, nitrogen and sulfur, litter for animal bedding and boat anchor. In addition, mangrove products are good sources of food for animals and of flokloric medicine for human beings. Morover, exellent firewood and certain chemicals such as tannin, methanol and acetic acid have been long known to be obtained from the plants in this area.

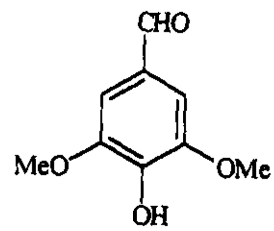
At present, many mangrove areas in Thailand have been destroyed and used for developing projects in many ways such as constructing houses, building factories and mine, fishery as well as shrimp and fish farms, and harbor construction.[3] From this activity made an effect to an environment including the vanishing of mangrove resources. In order to maximize the benefits from the utilization of mangrove plants before their extinction, one way is to study and to understand their natural products chemistry. The outcome from the investigation of their chemical constituents should present some informative data such as providing some promising medicinal and/or agrochemical substances.

Two basis concepts are established to justify the study of constituents of mangrove plants. The first one is that the mangrove plants are surrounding by under stressful conditions such as violent environment, high concentration of moisture and abundance of living organisms such as microorganisms and insects. Hence, the plants that can survive in this area should plausibly have a mechanism to protect themselves from microorganisms and insects. The second concept is those numerous mangrove plants have been used as folklore medicinal plants. The uses of *Acanthus* species to cure skin diseases and those of *Ceriops roxburgiana* to treat for suffering from diarrhea and vomiturition [4] are among these examples.

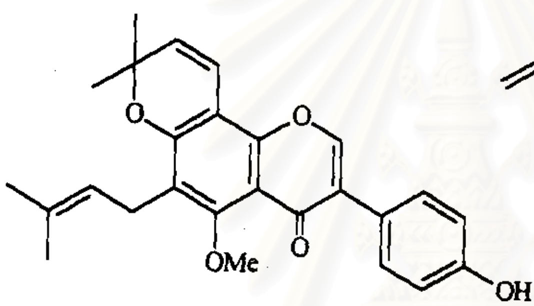
Up till now, the chemistry of natural products of mangrove plants has widely been studied. Several isolated substances were identified as novel compounds and a number of known compounds were reisolated and proved to possess biological activity. The occurance of biologically active substances positively supports the two abovementioned hypotheses. For instance, taraxerol (1) and syringaldehyde (2), isolated from *Rhizophora apiculata*, were found to exhibit as an antifungal and an antifeedant agent, respectively.[5] Some constituents of *Derris* genera were reported to reveal the fish toxicity such as rotenone (3). Moreover, scandonin (4) and warangalone (5) exhibited the pesticidal properties.[6] The roots of *Acanthus illicifolius* were investigated and found a new triterpenoidal saponin.[7] In addition, benzoxazolin-2-one (6) was found to exhibit various pharmacological activity.[8] Therefore, the concepts of studying the utilization of the mangrove plants based on the knowledge gained by natural products chemistry approach is worth considering.



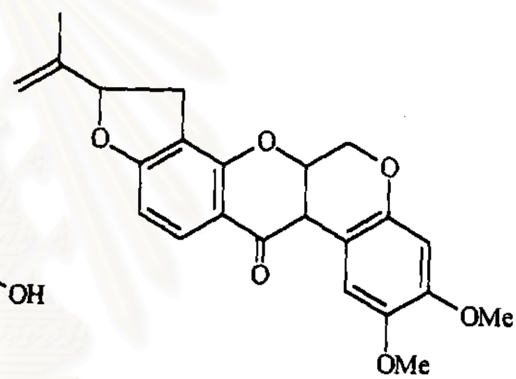
1 taraxerol



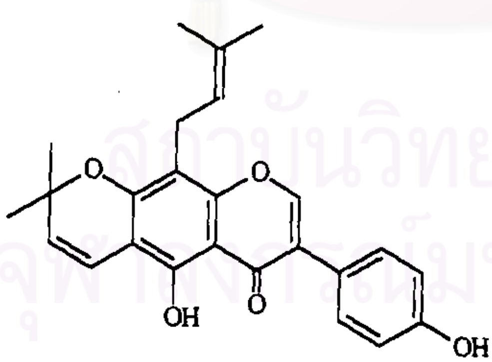
2 syringaldehyde



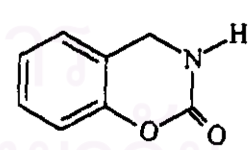
3 scandonin



4 rotenone



5 warangalone



6 benzoxazolin-2-one

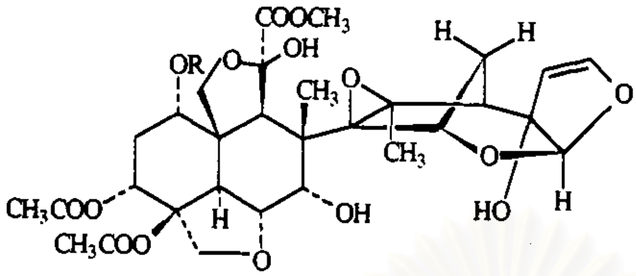
Fig. 1.1 Examples of some bioloactive compounds isolated from mangrove plants

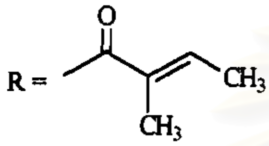
The Meliaceae family is an extremely large family including many genera such as, *Carapa*, *Cedrera*, *Khaya*, *Xylocarpus*, *etc*. The chemical constituents of Meliaceae have been well-known to involve a type of organic compound: limonoid, a group of chemically related bitter tetranortriterpenoid derivatives. Not only the interest on their structures of limonoids to organic synthetic chemists, but was a number of limonoids also proved to reveal a variety of biological activities.[9]

Among those attractive biological activities, an insect antifeedant and related activities against insects such as toxicity and repellant activity were found to be eminent. For example, a well-known limonoid, azadirachtin **(7)** isolated from *Azadirachta indica*, exhibited highly active insect antifeeding deterrent and growth regulator agent.[10] Gedunin **(8)**, a long known limonoidas an antifeedant substance and the corresponding compounds: fissinolide **(9)**, 7-deacetoxy-7-hydroxy gedunin **(10)**, 7-oxo-7-deacetoxy gedunin **(11)** were obtained from the separation of chloroform extract of the seeds of *Cabralea eichleriana.*[11]

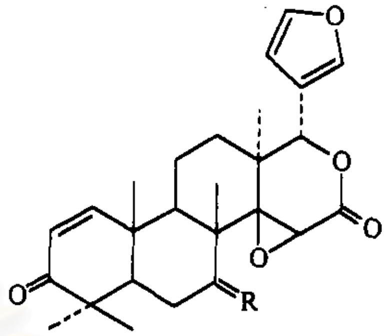
Other limonoids such as khivorin **(12)**, methyl angolensate **(13)**, andirobin **(14)** isolated from light petroleum extracts of the timbers of twenty species of Meliaceae, belonging to ten genera for instance, *Carapa*, *Guarea*, *Khaya*, *Trichilia*, *etc.*[12] A new limonoid glycoside **(15)** from the seeds of *Melia azedarach* showed antibacterial activity.[13] In addition, three azadarachins **(16-18)**, insect antifeedant limonoids, were isolated from the root barks of Chinese *Melia* *azedarach* Linn, along with six trichilins. All of them revealed antifeedant activity against the larvae of *Spodoptera exigua* Hubner.[14]

Moreover, the investigation of the stem barks of *Melia toosendan* led to the isolation of two new antifeedant limonoids, Trichilins I and J (19-20).[15] Recently, a preliminary screening study of some members of Mahogany family for insecticidal activity has been addressed. Two isolated limonoids (21,22) exhibited strong activity against the variegated cut worm *Peridroma saucia*.[16]





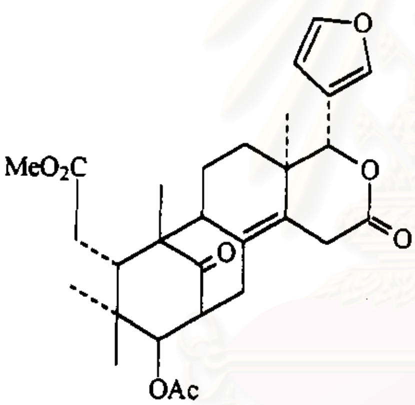
7 azadirachtin



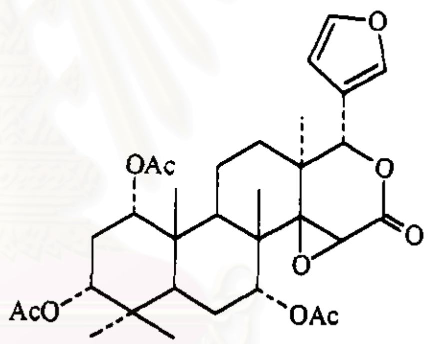
8 R = α-OAc, β = H, gedunin

10 R = α -OH, β = H, 7-deacetoxy-7-hydroxy gedunin

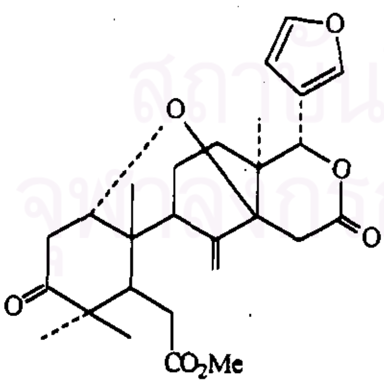
11 R = O, 7-oxo-7-deacetoxy gedunin



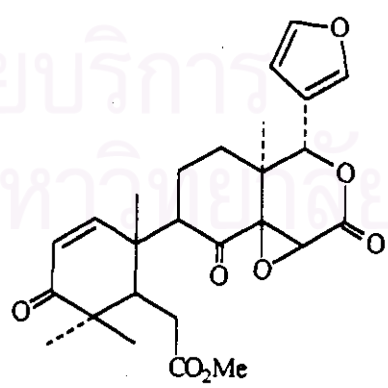
9 fissinolide



12 khivorin

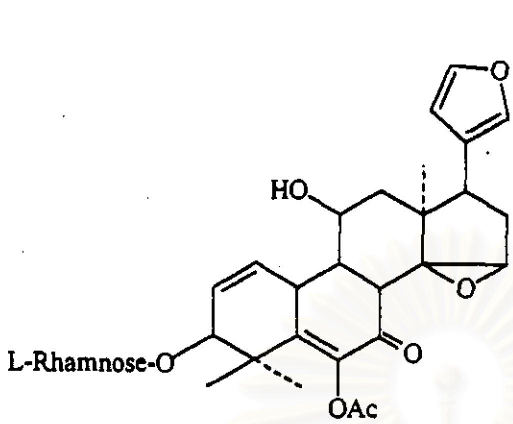


13 methyl angolensate

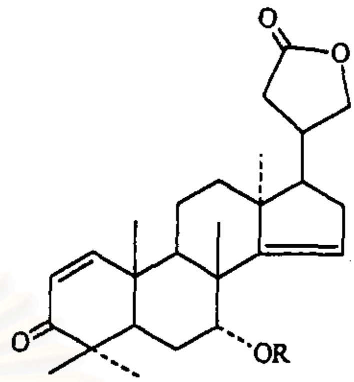


14 andirobin

Fig. 1.2 Some isolated compounds from the meliaceae plants

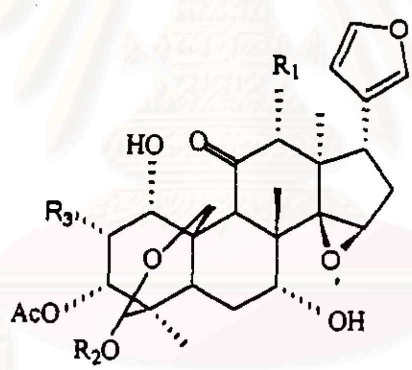


15 limonoid glycoside



21 R = Ac

22 R = H



16 R_1 = OH, R_2 = $COCH(CH_3)CH_2CH_3$, R_3 = H

17 R_1 = OAc, R_2 = $COCH(CH_3)CH_2CH_3$, R_3 = H

18 R_1 = OAc, R_2 = $COCH(CH_3)_2$, R_3 = H

19 R_1 = OAc, R_2 = $COCH(CH_3)CH_2CH_3$, R_3 = OAc, Trichilin I

20 R_1 = H, R_2 = $COCH(CH_3)CH_2CH_3$, R_3 = OAc, Trichilin J

Fig. 1.2 (continued)

In Thailand, the plants belonging to Xylocarpus genus are composed of 3 species, *i.e.*, *Xylocarpus gangeticus* Parkins. (Ta-bun), *Xylocarpus moluccensis* Koen. (Ta-bun-dum) and *Xylocarpus granatum* (Ta-bun-kao).[17] All of them are classified as mangrove plants. In this research, the fruits and the seeds of *Xylocarpus granatum* Koen., were selected to examine for their chemical constituents.

X. granatum (Ta-bun-kao) is a medium size tree. Their leaves are variable in size but tend to be quite large (about 5-12 cm. long by 2.5-6 cm. wide) and are oblong with a distinctly round tip. The small, whitish, typically unisexual flowers occurs on a somewhat irregular in floresence. The fruit is distinctive, being large (about 20 cm. in diameter), heavy (about 1 to 2 kg) brown and ball-shaped when mature. The fruit contains 4 to 17 semi-triangular seed. The bark is smooth, pale, greenish or yellowish typically peeling and flaking to present a blotchy appearance. The above ground root system is woody, flottened and snake-like. In additional well developed buttress may be formed.[18]

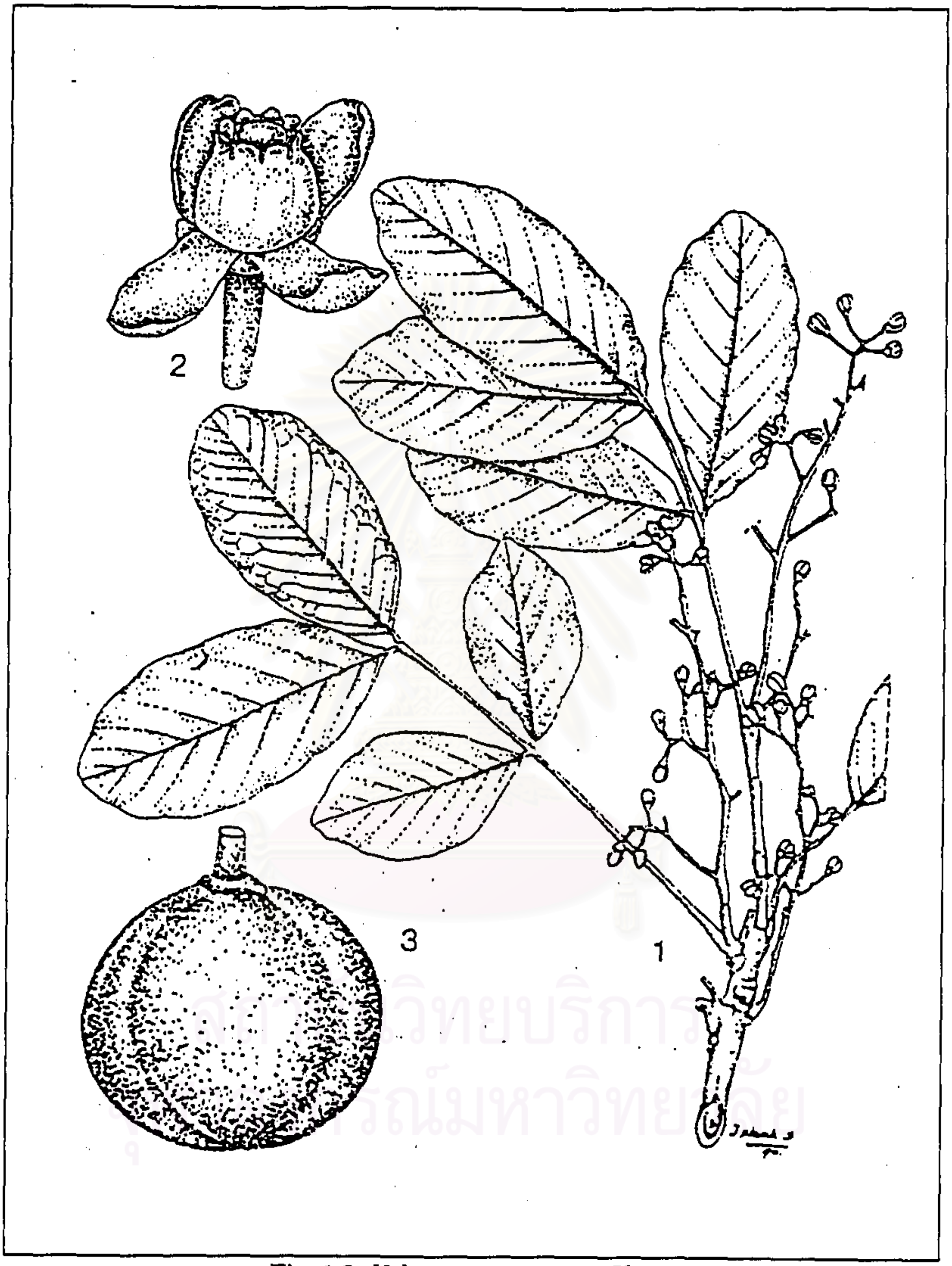


Fig. 1.3 *Xylocarpus granatum* Koen.

(1. flowering and branch, 2. female flower, 3. fruit)

Chemical Constituents Studies on Xylocarpus Genus

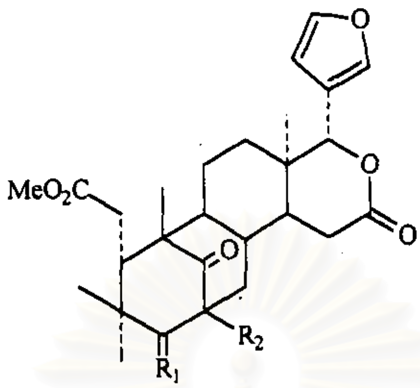
Literature surveys of chemical constituents of the plants belonging to Xylocarpus genus revealed that there have been a variety of organic substrates isolated (as tabulated in Table 1.1). The structures of some isolated compounds are shown in Fig. 1.4.

Table 1.1 Organic Compounds Found in Xylocarpus Genus

Scientific name	Plant parts	Crude extracts	Substance	Ref.
X. granatum Koen. (Tabun-kao)	timber	petroleum ether	gedunin **(8)**	19
	seed	petroleum ether	sitosterol, unidentified limonoid, destigloyl-6-deoxyswietenine acetate **(21)**, xylocarpin **(22)**, mexicanolide **(23)**, 7α-acetoxydihydronomilin **(24)**	19,23,26, 27
	root bark	methanol	*N*-methylflindersine **(25)**	22
		hexane	dihydrocheleryhrine **(26)**	
	fruit kernel	petroleum ether	xyloccsensin I, J **(27,28)**, glyceride ester **(29)**	26
	fresh leaves	chloroform -methanol	fatty acid, steriods, hydrocarbon	25

Table 1.1 (continued)

Scientific name	Plant parts	Crude extracts	Substance	Reference
X. molluccensis Koen. (Tabun-dum)	seed	2-methyl-pentane	xyloccensin A-F **(30-35)**	26
		hexane	2-hydroxy-distigloyl-6-deoxyswietenineacetate **(36)**, 7-oxo-7-deacetoxy gedunin **(9)**, humilin B **(37)**, new limonoid B **(38)**	21,24,27
	timber	hexane	xyloccensin G **(39)**, H **(40)**, I, A, B, E, F, destigloyl-6-deoxy swietenine acetate, angustidienolide **(41)**	25,23,27
	fruit	methanol	xylomollin **(42)**	27
	fruit kernel	petroluem ether	xyloccensin I, J **(27, 28)**	26
X. rumphii.	timber or seed		xyloccensins, phragmalin **(43)**, methylangolensate **(13)**	26

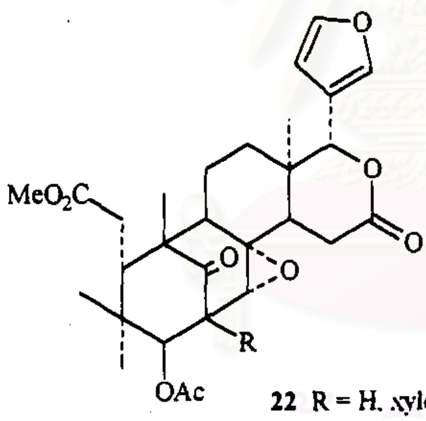


21 R_1 = H, α-OAc, R_2 = H; $\Delta^{8,30}$, destigloyl-6-deoxyswietenine acetate

23 R_1 = O, R_2 = H; $\Delta^{8,14}$, mexicanolide

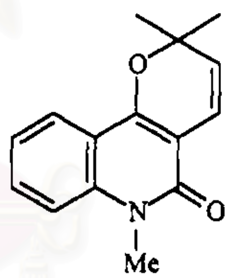
36 R_1 = H, α-OAc, R_2 = OH; $\Delta^{8,30}$, 2–hydroxy-destigloyl-6-deoxyswietenine acetate

41 R_1 = H, α-OAc, R_2 = H; $\Delta^{14,15}$, $\Delta^{8,30}$, angustidienolide

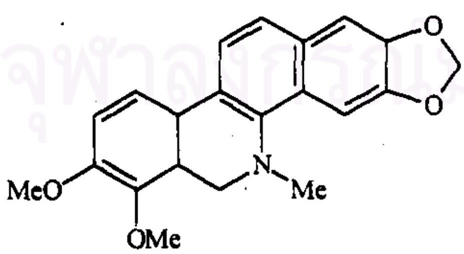


22 R = H, xylocarpin

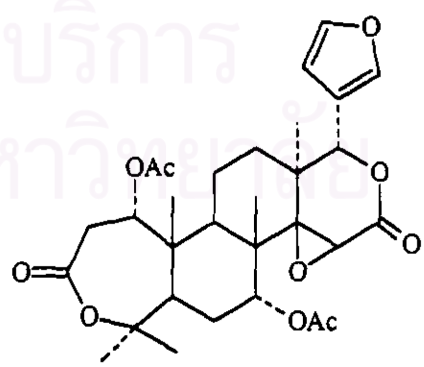
37 R = OH, humilin B



25 *N*-methylflindersine

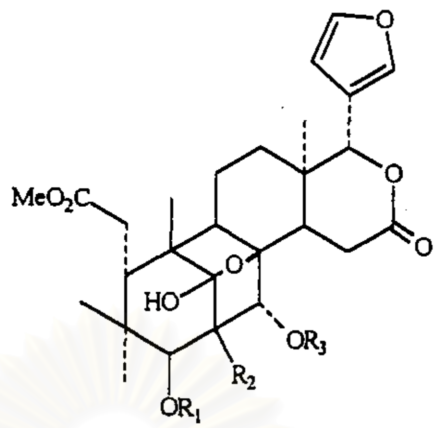


26 dihydrochelerythrine



24 7α-acetoxydihydronomilin

Fig. 1.4 Isolated compounds from *Xylocarpus* genus



27 R_1 = COMe, R_2 = OH, R_3 = COiBu, xyloccensin I

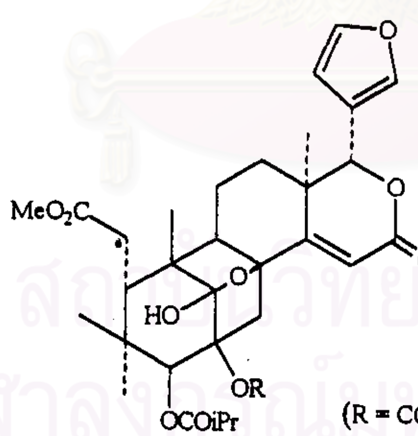
28 R_1 = COMe, R_2 = OH, R_3 = COiPr, xyloccensin J

30 $R_1 = R_3$ = COiPr or COiBu, R_2 = H; Δ 14,15, xyloccensin A

31 $R_1 = R_3$ = COiPr or COiBu, R_2 = H, xyloccensin B

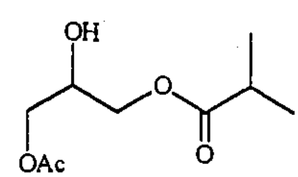
33 $R_1 = R_3$ = COiPr or COiBu, R_2 = OH; Δ 14,15, xyloccensin D

35 $R_1 = R_3$ = COiPr, R_2 = OH, xyloccensin F



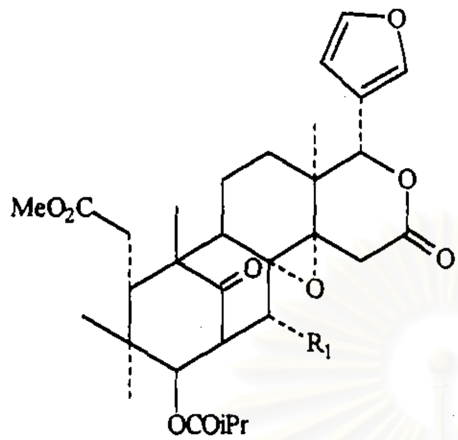
(R = COiPr or COiBu)

32 xyloccensin C

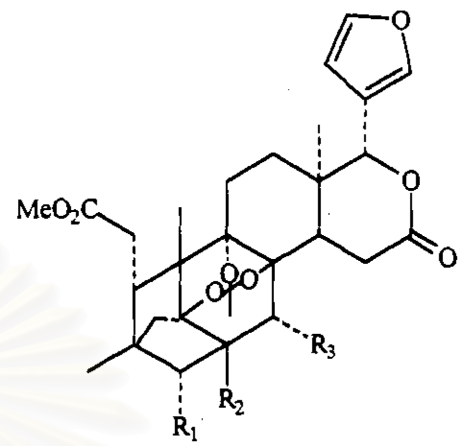


29 glyceride ester

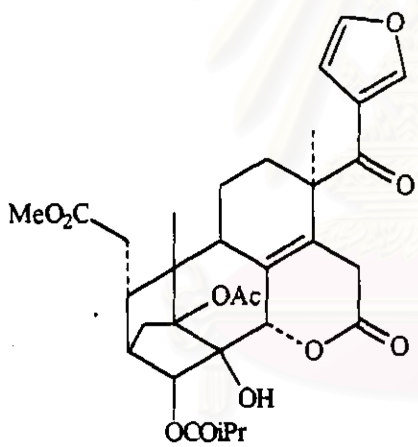
Fig. 1.4 (continued)



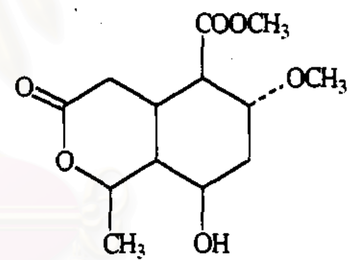
39 R_1 = OCOiPr, xyloccensin G
40 R_1 = H, xyloccensin H



34 $R_1 = R_2 = R_3$ = OAc, xyloccensin E
43 $R_1 = R_2 = R_3$ = H, phragmalin



38 new limonoid B



42 xylomollin

Fig. 1.4 (continued)

In 1970, Okorie and Taylor extracted timbers and seeds *of X. granatum* by refluxing with light petroleum.[19] Xylocarpin **(22)** was isolated and identified as the new compound, methyl 3β-acetoxy-8α,30α-epoxy-1-oxomeliacate. Gedunin **(8)**, antifungal substance was also isolated from this plant.

Kubo and co-workers studied unriped fruits of East African tree *X. moluccensis* in 1976 by extraction with methanol and isolated xylomollin **(42)**, which being an antifeedant against the African army worm *Spodoptera exampta*. In addition, this compound strongly inhibited the respiratory reactions of mitochondria from rat liver.[20] In the same year, Connolly and colleageus[21] examined the seeds and timbers of *X. moluccensis* by extraction with refluxing 2-methylpentane, from which new compounds, xyloccensins A, B, D and F **(30,31,33 and 35)** were isolated. These compounds are derivatives of methyl meliacate containing a 1,8-hemiacetal group. Xyloccensin E **(34)** is phragmalin triacetate.

In 1977, Chou and co-worker[22] isolated an insect antifeedant *N*-methylflindersine **(25)** from the root barks of *X. granatum*. Later, Ng[23] isolated 7 α-acetoxydihydronomiline from the seeds of *X. granatum* by stirring with methanol. Mexicanolide **(23)**, gedunin **(8)** and carapin **(22)** were also obtained from this methanolic extract.

In 1983 Taylor reported three new compounds, xyloccensins G, H and I **(39,40 and 27)** from *X. mollucensis* timbers by extraction with refluxing iso-hexane.[24] A structure for xyloccensin C was also deduced by this work.

In 1984, Hogg and Gillan [25] studied the constituents of the leaves from eleven species of mangrove. A mixture of fatty acids, sterols and hydrocarbons were reported as compositions of the leaves of *X. granatum*.

Alvi and colleageus[26] reported in 1991 for the isolation of two new limoniods, xyloccensin I and J **(27 and 28)** from the Fijian medicinal plants *X. granatum* and *X. mollucensis*.

In 1992, Mulholand and Taylor[27] extracted the timbers and seeds of *X. mollucensis* by refluxing with hexane. A rich mixture of limonoids was isolated. Some known substances from timbers were destigloyl-6-deoxyswietenine acetate **(21)**, angustidienolide **(41)**, phragmalin **(43)** and xyloccensin E **(34)**. An extraction of the seeds of the same species led to the isolation of 2-hydroxydestiglyl-6-deoxyswietenine acetate **(36)**, 7-oxo-deacetoxy gedunin **(9)**, 2-hydroxyfissinolide as a new limoniod, B_3 **(38)**.

The Goal of This Research

A preliminary study involving cooperative research between Natural Products Research Unit, department of chemistry and Bee Biological Research Unit, department of biology, Chulalongkorn University with the aim to screen for bioloactive compounds from Thai mangrove plants showed that the ethanolic extract of the fruits of *X. granatum* gave a high level of feeding inhibition against Greater Wax Moth (*Gallerlia mellonella*). This attractive preliminary result called

Therefore, the goal of this research can be summarized as follows:

1. To extract and to isolate the organic constituents from the fruits of *X. granatum*
2. To elucidate the structural formulae of the isolated substances
3. To search for the bioactive compounds that can be used as an antifeedant agent against *Gallerlia mellonella* and/or otherwise by using bioassay results as a guide.