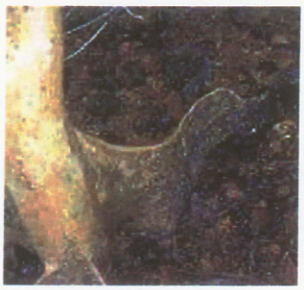
1 INTRODUCTION

1.1 Introduction

Xylocarpus granatum Koenig, a mangrove plant belonging to the Meliaceae family, local names in Thai e.g. Tabun khao (ตะบูนขาว), Tabun (ตะบูน) and Krabun khao (กระบูนขาว) (The Forest Herbarium Royal Forest Department, 2001). It occurs in mangrove swamps on the ocean coast, and in the estuaries of tidal rivers, and ranges from East Africa to the Pacific Islands, appearing quite the same in East Africa and in Queensland, where it extends as far south as Cairns (Mulholland, *et al.*, 1992). The family Meliaceae is known to contain about 1400 species. In Thailand, the plants belonging to *Xylocarpus* genus are composed of 3 species, *i.e.*, *X. granatum*, *X. moluccensis* and *X. rumphii* (The Forest Herbarium Royal Forest Department, 2001).

Xylocarpus granatum is a tree, 3-8 meter tall; buttresses are long and snaking laterally; the bark is smooth, unfissured and thin, displaying a pattern of light-brown to yellowish or greenish patches, caused by peeling of the bark. Leaves pinnate, leaflets bright light green when young, dark green when old, narrowly drop-shaped, with rounded tips, and on average 10 cm long and 4 cm wide. Petiole of the leaf is short and corky. The flowers of *X. granatum* are white in color and very small about 8 mm across; capsule woody, A big fruit has a size ranging from a big orange to grapefruit, characterised by a hard, heavy and lignified capsule, in which several seeds are enclosed leading to the common name of the "cannon ball tree". The above ground root system is often absent in young individuals.Older individuals often display butresses, which cause enlargement of trunk base diameter, and which extend into partially above-ground ribbon like roots.

Habitat of
Xylocarpus granatum



Plank buttresses
showing the snake-like

Bark

Fruiting branch

Fruits in various views
showing seeds within

Seeds

Flowering branch

Flowers

Xylocarpus granatum
tree

Figure 1 *Xylocarpus granatum* (Meliaceae)

1.2 *Review of Literatures*

According to information from NAPRALERT database developed by University of Illinois at Chicago, several types of compounds present in plants of *Xylocarpus* genus can be classified into groups as follows:

1. Carbohydrates
2. Glyceride ester
3. Isoquinoline alkaloids
4. Quinoline alkaloids
5. Secoiridoid monoterpenes
6. Steroids
7. Triterpenes

These compounds are presented in **Table 1.**

Table 1. Compounds isolated from *Xylocarpus* genus

1 = Carbohydrates **2** = Glyceride ester **3** = Isoquinoline alkaloids

4 = Quinoline alkaloids **5** = Secoiridoid monoterpenes **6** = Steroids

7 = Triterpenes

Scientific name	Investigated Part	Compound	Bibliography
X. granatum	Fruits	Xyloccensin K, **7a**	Kokpol, *et al.*, 1996
	Heartwoods	Gedunin, **7b**	Sundarasivarao, *et al.*, 1977
	Kernels	1-Iso-butyrate 3-acetoxy propan-2,3-diol, **2a** Xyloccensin I, **7c** Xyloccensin J, **7d**	Alvi, *et al.*, 1991
	Leaves	Fructose, **1a** Glucose, **1b** Sucrose, **1c**	Apopp, 1984
	Rootbarks	Acetonyldihydrochelerythrine, **3a** Dihydrochelerythrine, **3b** N-methylflindersine, **4a**	Chou, *et al.*, 1977
	Seeds	7α-acetoxydihydronomilin, **7e** Detigloyl-6-deoxyswietenin acetate, **7g**	Ng and Fallis, 1979 Okorie and Taylor,1970

Table 1 (Continued)

Scientific name	Investigated Part	Compound	Bibliography
X. granatum	Seeds	Gedunin, **7b** Mexicanolide, **7f** *β*-Sitosterol, **6a** Xylocarpin, **7h** Xylomollin, **5a**	Okorie and Taylor,1970 Ng and Fallis, 1979 Okorie and Taylor,1970 Ng and Fallis, 1979
	Timber	Detigloyl-6-deoxyswietenin acetate, **7g** Gedunin, **7b** Xylocarpin, **7h**	Mulholland and Taylor, 1992
	Woods	Gedunin, **7b**	Taylor, *et al.*, 1965
X. moluccensis	Barks	Acetonyldihydrochelerythrine, **3a** N-methylflindersine, **4a** N-norchelerythrine, **3c**	Chou and Nakanish,1977
	Fruits	Xylomollin, **5a**	Hassam and Hutchinson, 1980 Kubo, *et al.*, 1976
	Kernels	1-Iso-butyrate 3-acetoxy propan-2,3-diol, **2a** Xyloccensin I, **7c** Xyloccensin J, **7d**	Alvi, *et al.*, 1991

Table 1 (Continued)

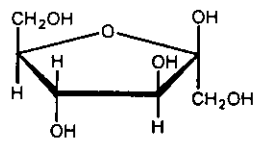
Scientific name	Investigated Part	Compound	Bibliography
X. moluccensis	Seeds	7-Oxogedunin, **7i** 2-Hydroxyfissinolide, **7j** 2-Hydroxy-detigloyl-6-deoxyswietenin acetate, **7k**	Mulholland and Taylor, 1992
	Timber	Detigloyl-6-deoxyswietenin acetate, **7g** Angustidienolide, **7l** 7-Oxogedunin, **7i** Xyloccensin G, **7m** Xyloccensin H, **7n** Xyloccensin I, **7c**	Mulholland and Taylor, 1992 Taylor, 1983
	Woods	Angustidienolide, **7l** β-Sitosterol, **6a** 6β-Hydroxystigmast-4-en-3-one, **6b** Detigloyl-6-deoxyswietenin acetate, **7g** Stigmasterol, **6c**	Mulholland and Taylor, 1992 Bercich, *et al.*, 1998 Mulholland and Taylor, 1992 Bercich, *et al.*, 1998

Table 1 (Continued)

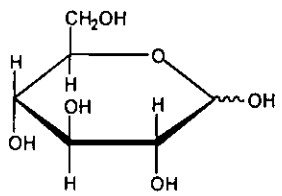
Scientific name	Investigated Part	Compound	Bibliography
X. moluccensis	Woods	2,3,30-Triacetatephragmalin, **7o** 3,30-Diacetatephragmalin, **7p**	Mulholland and Taylor, 1992

Structures

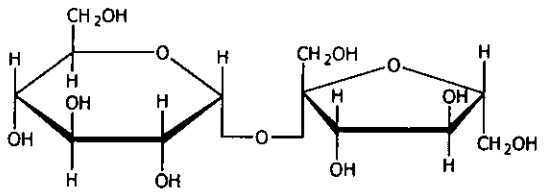
1. Carbohydrates



1a : Fructose

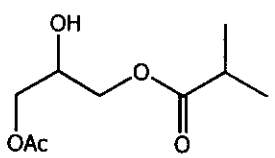


1b : Glucose



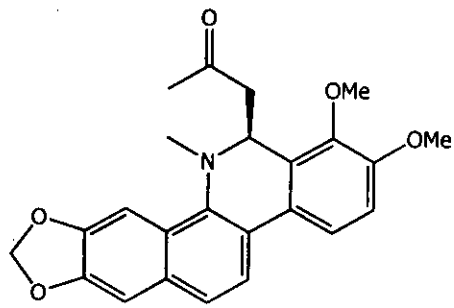
1c : Sucrose

2. Glyceride ester

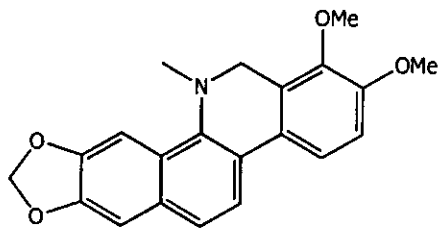


2a : 1-Iso-butyrate-3-acetoxy propan-2,3-diol

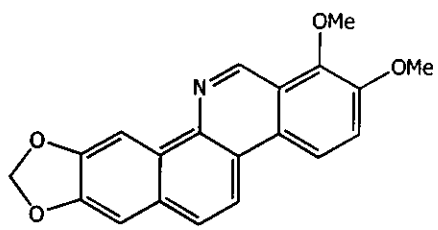
3. Isoquinoline alkaloids



3a : Acetonyldihydrochelerythrine

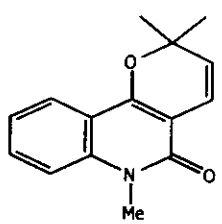


3b : Dihydrochelerythrine



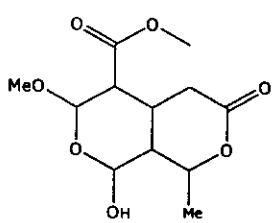
3c : N-Norchelerythrine

4. Quinoline alkaloids



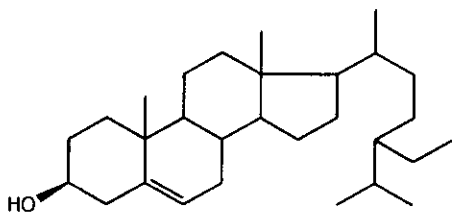
4a : N-Methylflindersine

5. Secoiridoid monoterpenes

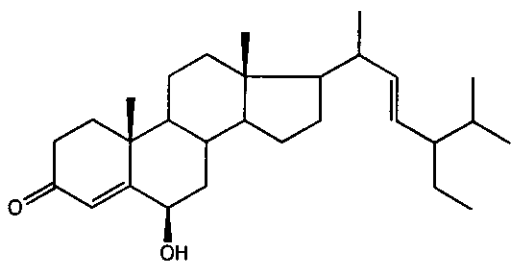


5a : Xylomollin

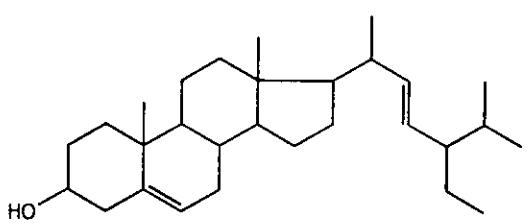
6. Steroids



6a : β-Sitosterol

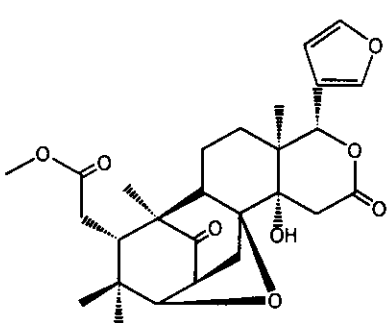


6b : 6β -Hydroxystigmast-4-en-3-one

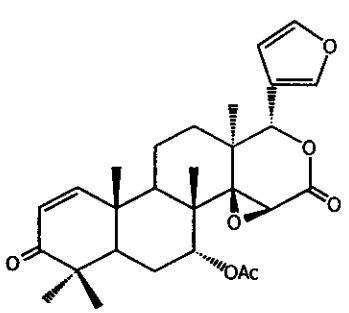


6c : Stigmasterol

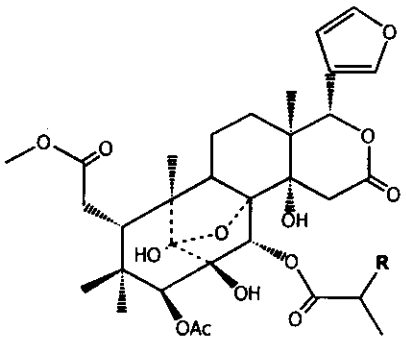
7. Triterpenes



7a : Xyloccensin K

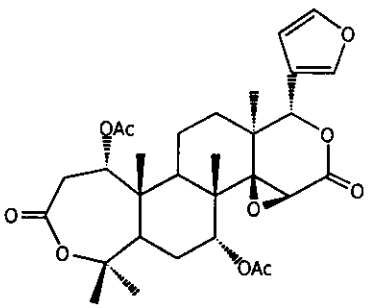


7b : Gedunin

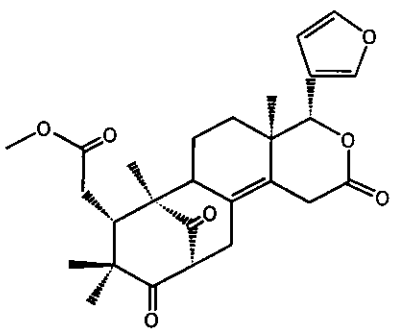


7c : R = Et; Xyloccensin I

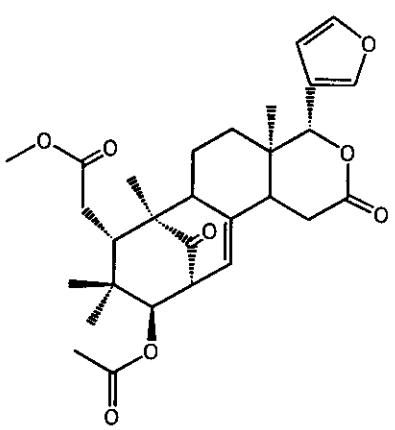
7d : R = Me; Xyloccensin J



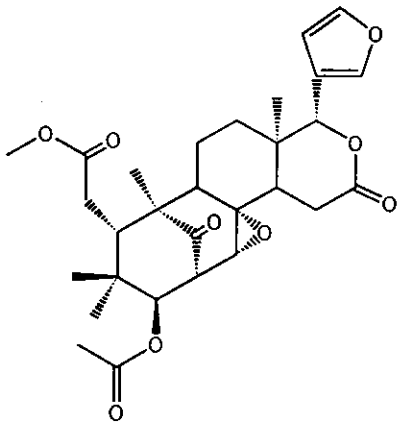
7e : 7α-Acetoxydihydronomilin



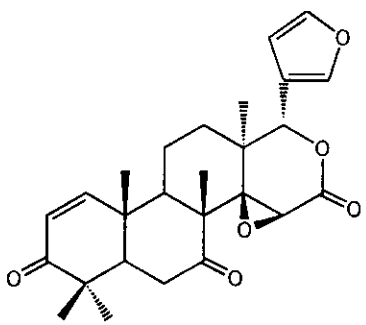
7f : Mexicanolide



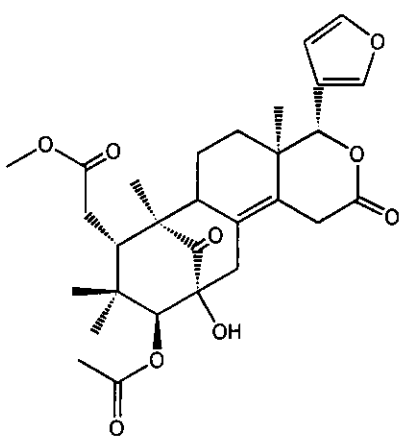
7g : Detigloyl-6-Deoxyswietenin



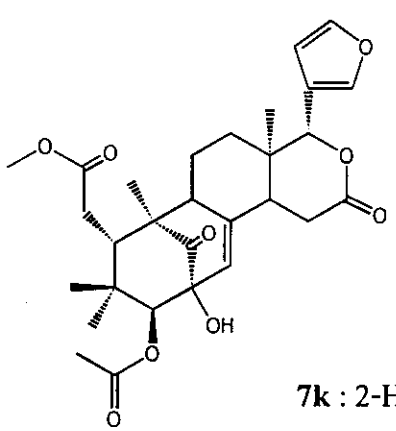
7h : Xylocarpin



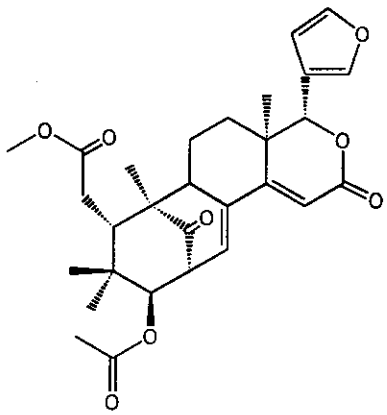
7i : 7-Oxogedunin



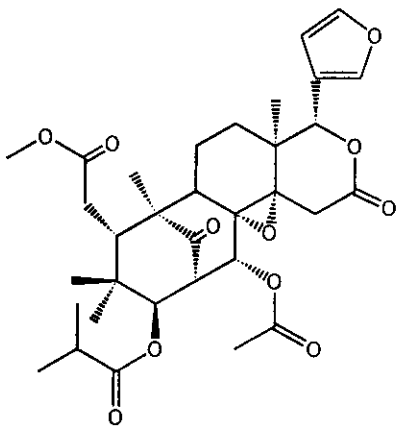
7j : 2-Hydroxyfissinolide



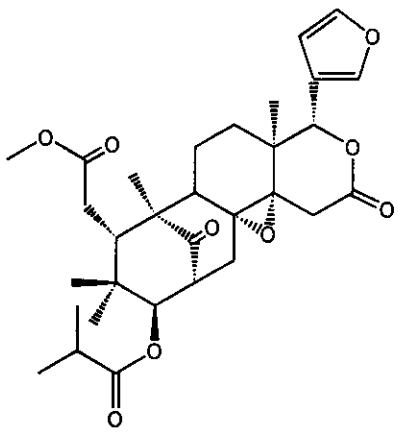
7k : 2-Hydroxy detigloyl-6-deoxyswietenin acetate



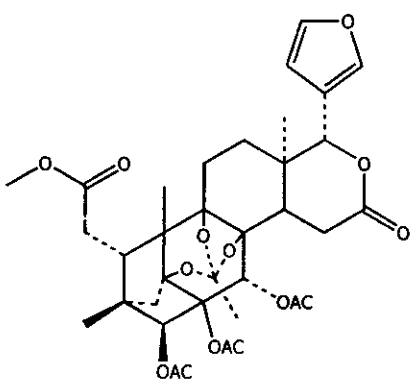
7l : Angustidienolide



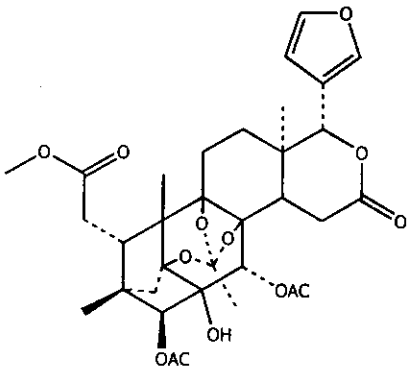
7m : Xyloccensin G



7n : Xyloccensin H



7o : 2,3,30-Triacetatephragmalin



7p : 3,30-Diacetatephragmalin

1.3 Biosynthesis of Limonoid

The order Rutales, including the families Rutaceae, Meliaceae, Simaroubaceae and Cneoraceae, is among the richest and most diverse sources of secondary metabolites in the Angiospermae. Almost every part of the trees, leaves, barks and seeds have long been used for medicinal and plant protection purposes. The most characteristic metabolites of the Rutales are limonoids, which are tetranortriterpenoids derived from tirucallane (H-20α) or euphane (H-20β) triterpenoids with a 4,4,8-trimethyl-17-furanylsteroidal skeleton. They have also attracted considerable interest because of their fascinating structural diversity and their wide range of biological activity. In particular, they characterize members of the family Meliaceae, where they are abundant and varied.

Limonoids are thought to be derived from the tetracyclic triterpenoids tirucallane (20-*S*, H-20α) or euphane (20-*R*, H-20β). Many triterpenoids with the same ring structure have been observed in meliacean plants. Modification of the parent triterpene skeleton takes the form of oxidation, often with ring fission and recyclization involving any of the four rings, to give heterocyclic systems. According to the generally accepted scheme, the Δ^7-bond is epoxidized to a 7-epoxide, which is

then opened, including a Wagner-Meerwein shift of the 14-Me to C-8, formation of the 7-OH, and introduction of a double bond at C-14/C-15.

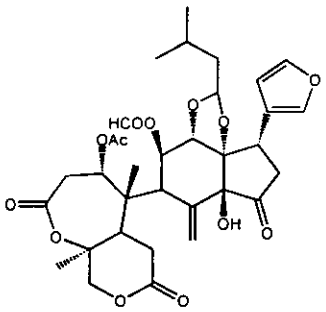
Cyclization in the side chain most commonly proceeds through oxidation of the C-21 methyl and leads to a 21/23 or, more rarely, 21/24 oxides, from which the 17α-furan ring is formed with the loss of four carbons – a possible pathway is demonstrated in Figure 1.1. The last step is accomplished after formation of the 4,4,8-trimethylsteroid skeleton, as indicated by the occurrence of several protolimonoids with an intact C_8 side chain.

The wide variety of structural types found among the limonoids is generally the product of further oxidative ring opening and skeletal rearrangement (Figure 1.2). The rings A and D are sometimes oxidized to lactones (A and D-seco limonoids) by Baeyer-Villiger oxidation of the C-3 and C-16 Keto groups. A,D-seco limonoids are found in all families of Rutales, especially in the Rutaceae.

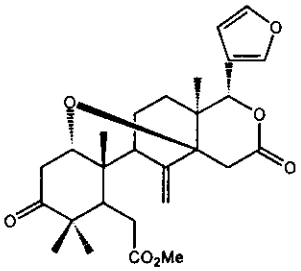
In the Meliaceae the degree of complexity in limonoid structures becomes greater, with fission of rings B and C occurring widely, as well as the rings A and D modifications mainly found in the Rutaceae. In particular, synthesis of the C-seco limonoids occurs only in the Meliaceae belonging to the tribe Meliaceae, in which they appear to be restricted to the genera *Melia*. The biosynthetic origin of the C-seco limonoids is less clear, because there is controversy about the mechanism by which the C-12/C-13 bond of the C-ring is opened. One possible process involved, which includes introduction of an oxygen function at C-12, followed by oxidation to a ketone. Cleavage of the C-12/C-13 bond is accompanied by the simultaneous opening of a 14,15-epoxide to generate the CHO-12 and allylic 15-OH functions. Rotation about the C-8/C-14 bond would allow the 15-OH to recyclize with CHO-12 to form a lactol C-ring.

Commonly, the B-seco compounds are observed in nature accompanying fission of any of the rings A and D of the original limonoid skeleton. Rohitukin and

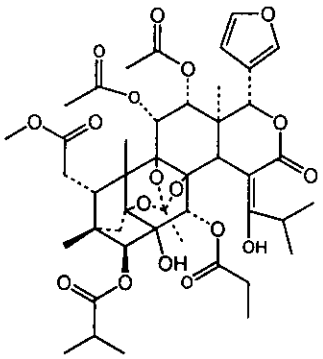
methyl angolensate are typical compounds and the latter may be extensively rearranged to yield a complex phragmalin-type limonoid such as bussein.



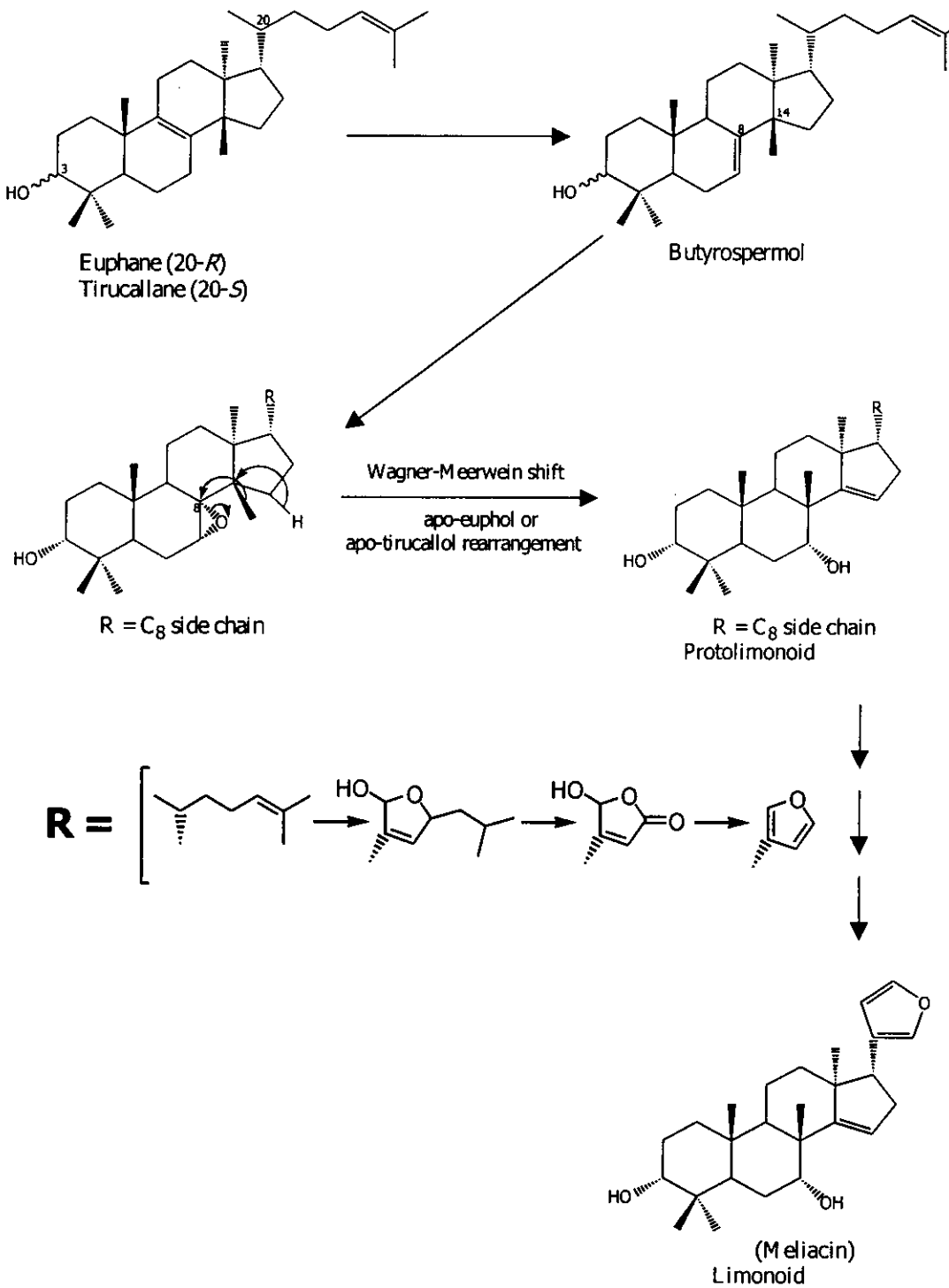
Rohitukin



Methyl angolensate

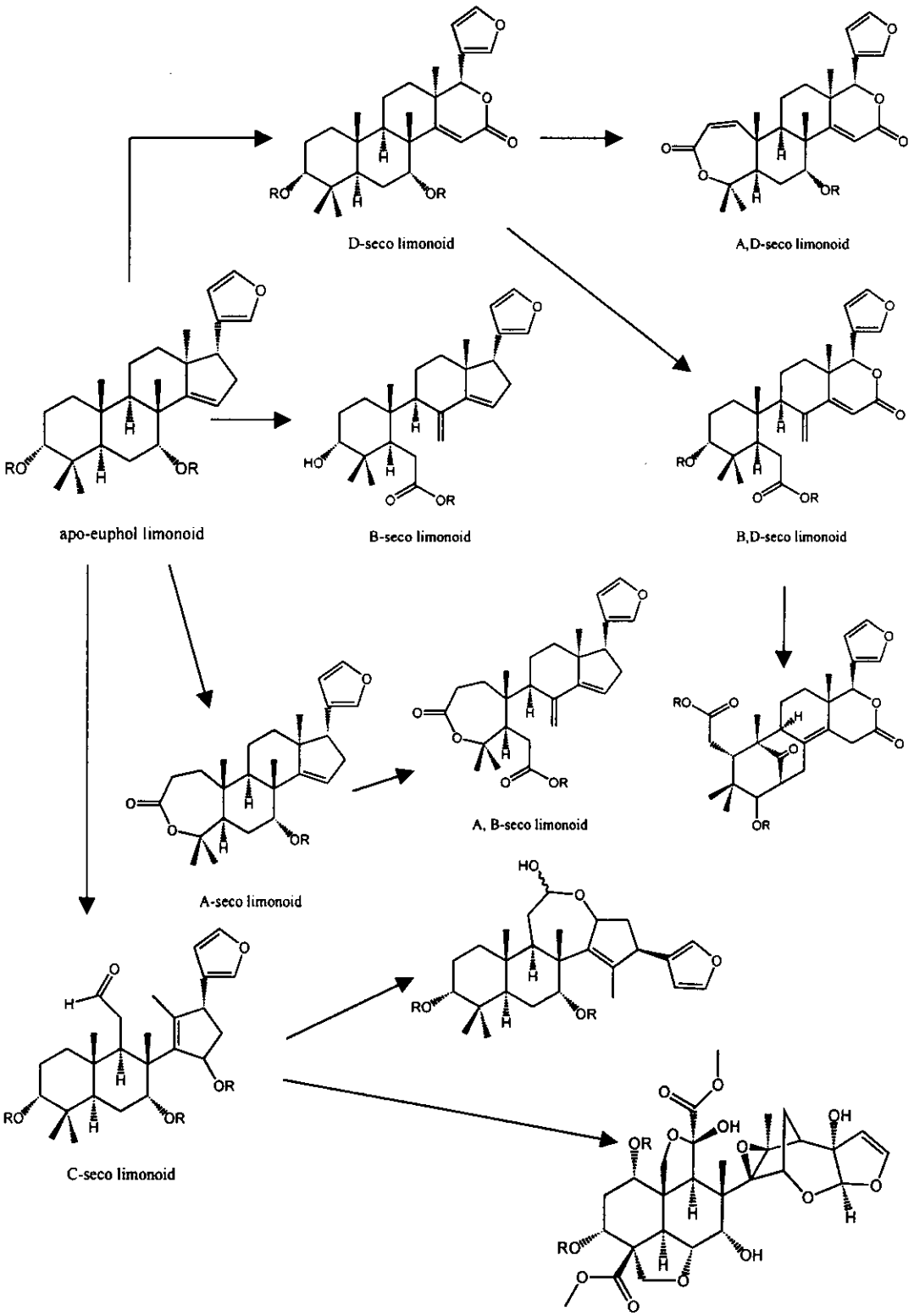


Bussein

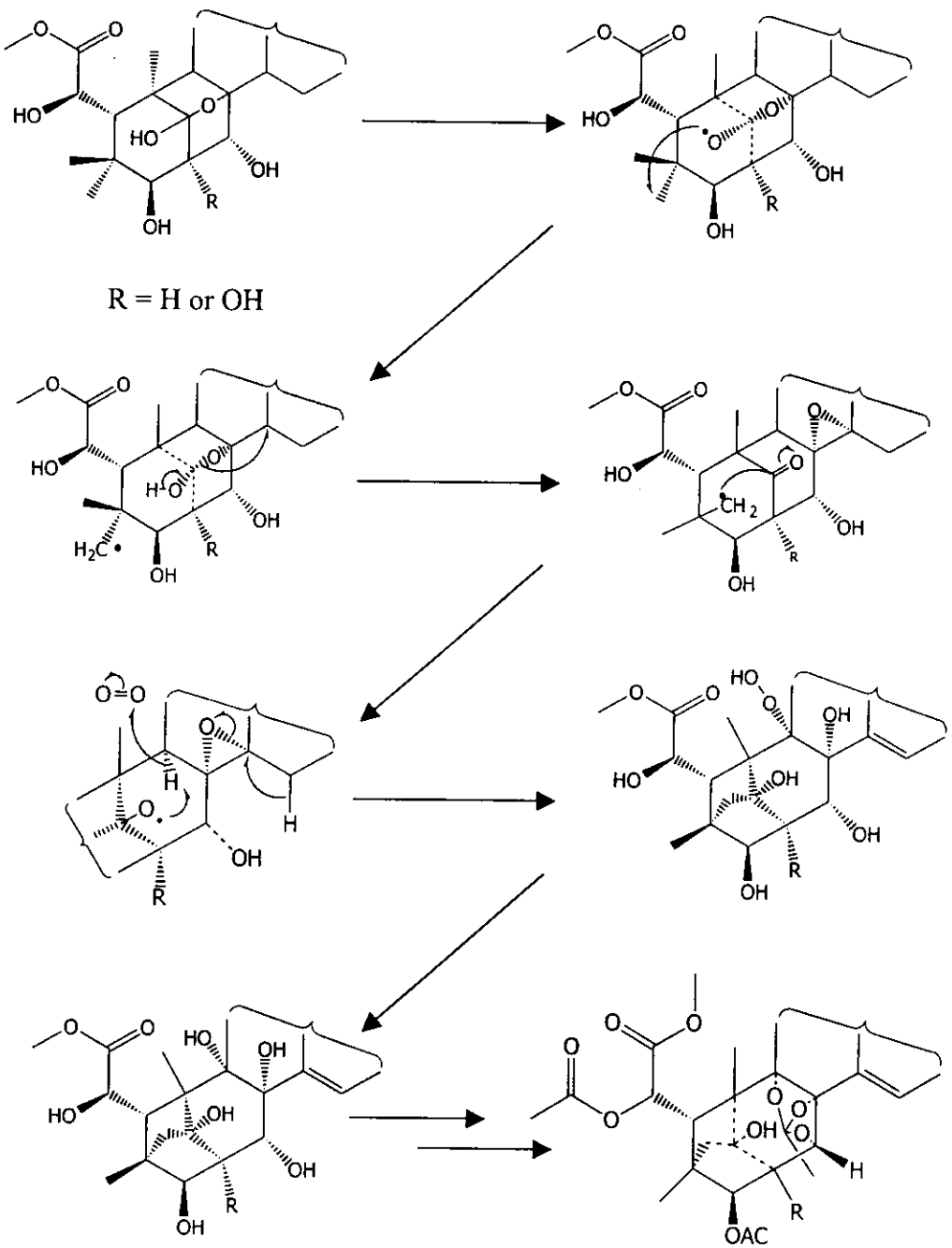


Scheme 1 Biosynthetic pathway leading to the formation of a simple limonoid.

(Champagne, *et al*,1992)



Scheme 2 Major biosynthetic routes of limonoids. (Champagne, *et al*, 1992)



Scheme 3 Proposed Biosynthetic Pathway to 8,9,30-Phragmalin Orthoesters from a Mexicanolide. (Wu, *et al*, 2004)

1.4 Biological Activity of Xylocarpus Species

Many limonoids have a bitter taste. A number of Citrus juices develop a bitter taste gradually after the immediate bitterness due to the flavanone glycoside. The wide occurrence of the limonoids in nature has evoked considerable interest in their biological activity. Recent work has established a wide range of biological activities for these compounds, including insecticidal, insect antifeedant and growth-regulating properties, a variety of medicinal effects in animals and humans, and antifungal, bactericidal and antiviral activities. The range of biological activity encountered in the limonoids has been reviewed (Champagne, *et al.*, 1992).

The importance of *Xylocarpus* in traditional medicine throughout the tropical world is apparent from NAPRALERT database. The significant biological activities of the extract of *Xylocarpus* species are summarized in **Table 2** and the importance ethnomedical applications are summarized in **Table 3**.

Table 2 Ethnomedical information on *Xylocarpus*

Plant	Ethnomedical	Referenec
X. benadirensis	Aphrodisiac	Watt and Breyer-Brandwijk, 1962
X. granatum	KAHI Aphrodisiac	Singh, *et al.*, 1984 Kokwaro, 1976
X. moluccensis	Aphrodisiac	Kubo, *et al.*, 1976
X. obovatus	Dysentery	Wasuwat, 1967
X. species	Aphrodisiac	Waddell, *et al.*, 1980

Table 3 Biological activities for extracts of *Xylocarpus granatum*

Part	Test activity	Crude extract	Activity	Reference
Fruits	Antibacterial activity (*Bacillus subtilis*)	EtOH	Inactive	Taniguchi, *et al.*, 1978
	Antibacterial activity (*Escherichia coli*)	EtOH	Inactive	
	Antifungal activity (*Penicillium crustosum*)	EtOH	Inactive	
	Antiyeast activity (*Saccharomyces cerevisiae*)	EtOH	Inactive	
	Platelet activating factor receptor binding inhibition	MEOH	Inactive	Jantan, *et al.*,1996
Leaves	Insect repellent activity (*Aedes aegypti*)	Acetone	Active	Thangam and Kathiresan, 1993
	Antifilarial activity (*Brugia malayi*)	H_2O	Active	Zaridah, *et al.*, 2001
	Platelet activating factor receptor binding inhibition (binding inhibition 33%)	MeOH	Weak Activity	Jantan, *et al.*, 1996
Leaves + Stems	Reverse transcriptase inhibition (11% inhibition vs. HIV-1 reverse transcriptase)	MeOH	Weak Activity	Tan, *et al.*, 1991

Table 3 (Continued)

Part	Test activity	Crude extract	Activity	Reference
Roots (Rootbark)	Antibacterial activity (*Bacillus subtilis*)	EtOH	Active	Taniguchi, *et al.*, 1978
	Antibacterial activity (*Diplococcus pneumoniae*)	EtOH	Active	Chou, *et al.*, 1977
	Antibacterial activity (*Escherichia coli*)	EtOH	Inactive	Taniguchi, *et al.*, 1978
	Antibacterial activity (*Proteus vulgaris*)	EtOH	Active	Chou, *et al.*, 1977
	Antibacterial activity (*Staphylococcus aureus*)	EtOH	Active	
	Antifungal activity (*Penicillium crustosum*)	EtOH	Active	Taniguchi, *et al.*, 1978
	Antifungal activity (*Rhizopus delemay*)	EtOH	Active	Chou, *et al.*, 1977
	Antifungal activity (*Trichophyton mentagrophytes*)	EtOH	Active	
	Antiyeast activity (*Candida albicans*)	EtOH	Active	
	Antiyeast activity (*Saccharomyces cerevisiae*)	EtOH	Active	Taniguchi, *et al.*, 1978

Table 3 (Continued)

Part	Test activity	Crude extract	Activity	Reference
Seeds	Antifilarial activity (*Brugia malayi*)	H_2O	Active	Zaridah, etal., 2001
Seed husks	Antifilarial activity (*Brugia malayi*)	H_2O	Weak Activity	Zaridah, etal., 2001
Wood	Platelet activating factor receptor binding inhibition (binding inhibition 23%)	MeOH	Weak Activity	Jantan, etal., 1996

This research involved isolation, purification and structure elucidation of chemical constituents isolated from the seeds of *X. granatum*. It is a part of the basic research on the utilization of Thai plant for pharmaceutical purposes.