

**The Study of Bioactive Compounds from
*Cratoxylum cochinchinense***

By

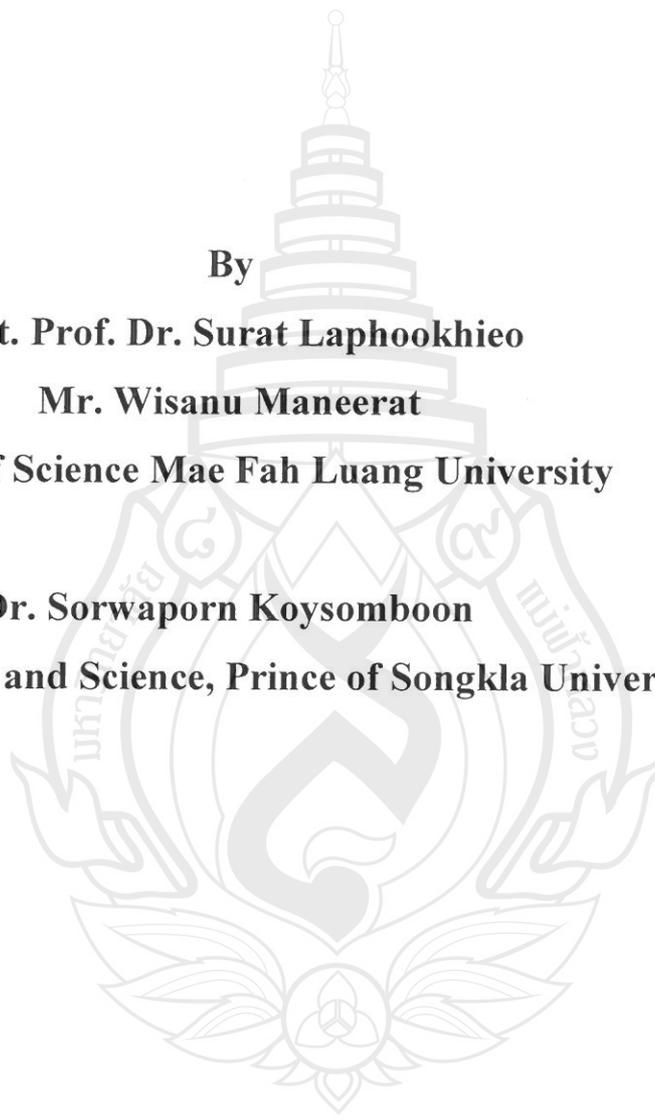
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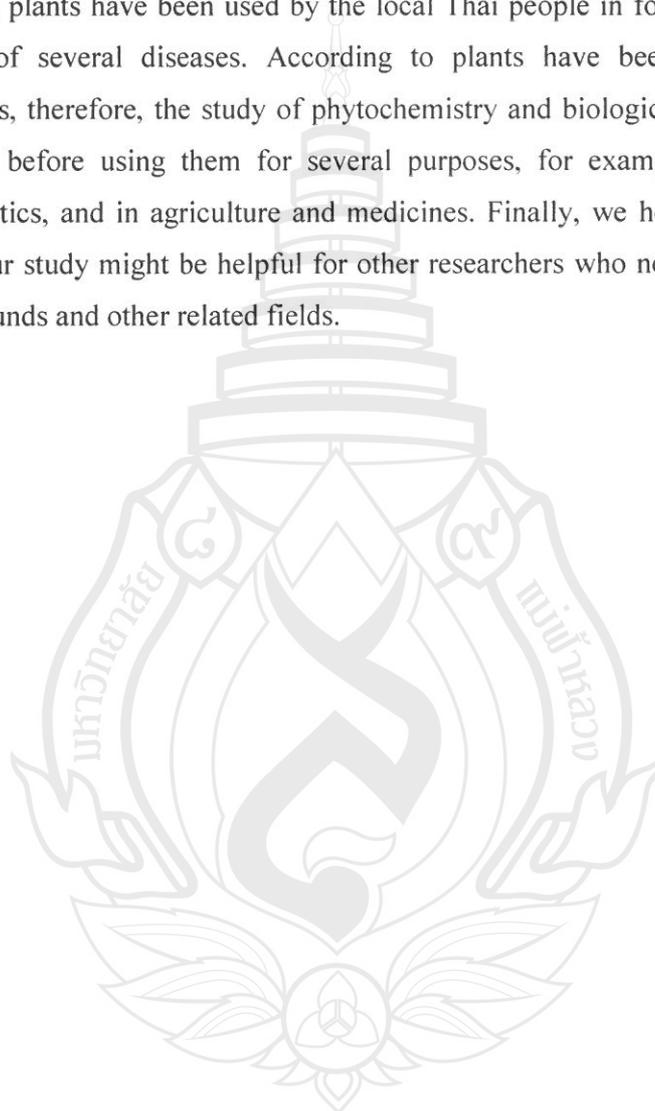
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2008

PREFACE

Plants have been used worldwide in traditional medicines for the treatment of diseases. It is estimated that even today approximately two-thirds to three-quarters of the world's population rely only on medicinal plants as their primary source of medicines. In Thailand, several plants have been used by the local Thai people in folk medicine for the treatment of several diseases. According to plants have been used for traditional medicines, therefore, the study of phytochemistry and biological activities are very important before using them for several purposes, for example as food supplements, cosmetics, and in agriculture and medicines. Finally, we hope that the information from our study might be helpful for other researchers who need to study on bioactive compounds and other related fields.



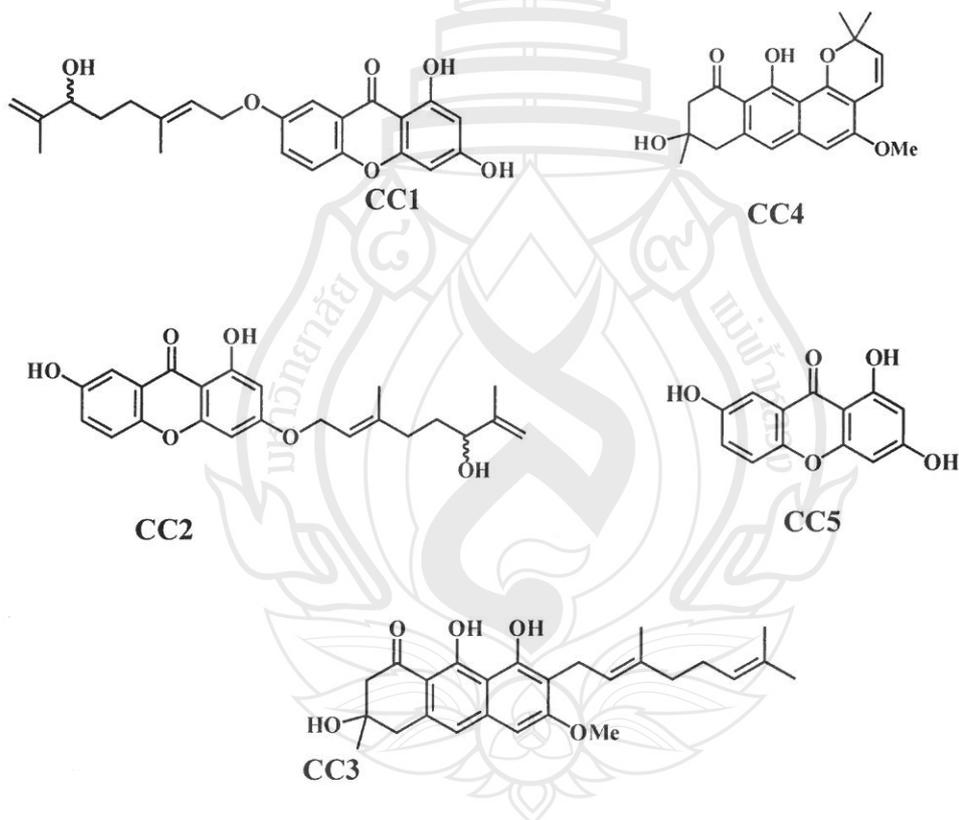
ACKNOWLEDGMENT

We are very grateful to Assoc. Prof. Dr. Chatchanok Karalai and Assoc. Prof. Chanita Ponglimanont, Department of Chemistry, Faculty of Science, Prince of Songkla University, for supporting some scientific materials. Special thanks are address to Assoc. Prof. Dr. Uma Prowat, Department of Chemistry, Faculty of Science and Technology, Phuket Rajabhat University for supporting NMR spectral data. We also thank Mae Fah Luang University for financial support.



บทคัดย่อ

การศึกษาส่วนสกัดเฮกเซนและเอทิลอะซิเตตของผลดิบกล้วยสามารถแยกสารประกอบแซนโทนชนิดใหม่ได้ 2 สารคือ cochinxanthone A (CC1) และ B (CC2) และสารประกอบที่ได้มีการรายงานมาแล้ว 3 สาร (CC3-CC5) โครงสร้างของสารประกอบทุกชนิดวิเคราะห์ด้วยข้อมูล 1D และ 2D NMR นอกจากนี้สารประกอบ CC3 และ CC4 ยังแสดงฤทธิ์ต้านมะเร็งปอด มะเร็งในช่องปาก มะเร็งทรวงอก และ ต้านเชื้อที่ก่อให้เกิดโรคมาลาเรียในเซลล์ทดลองในระดับดี



ABSTRACT

The investigation of hexane and EtOAc extracts of the fruits of *Cratoxylum cochinchinense* led to the isolation and identification of two new xanthenes (**CC1** and **CC2**) named cochinxanthone A and B along with three known compounds (**CC3-CC5**). All isolates were characterized using 1D and 2D NMR spectral data. Compounds **CC3** and **CC4** were evaluated for anti-cancer activity against NCI-H187 (human lung cancer), KB (oral human epidermal carcinoma) MCF-7 (breast cancer) cancer cell lines and antimalarial activity against *P. falciparum*.

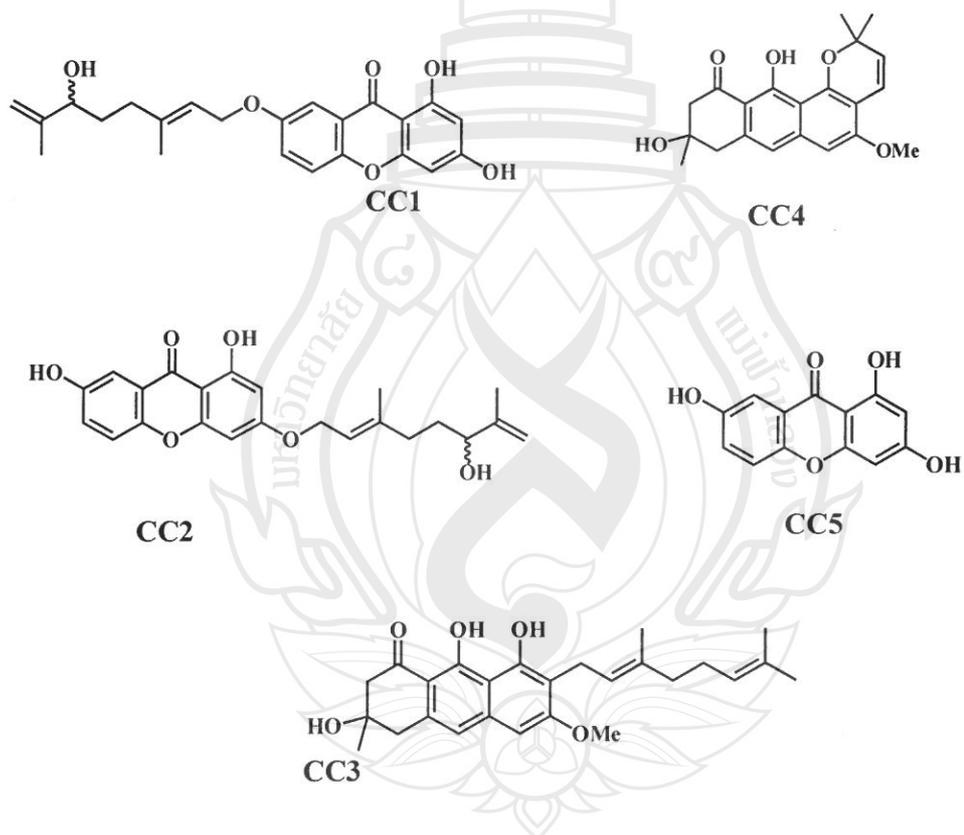


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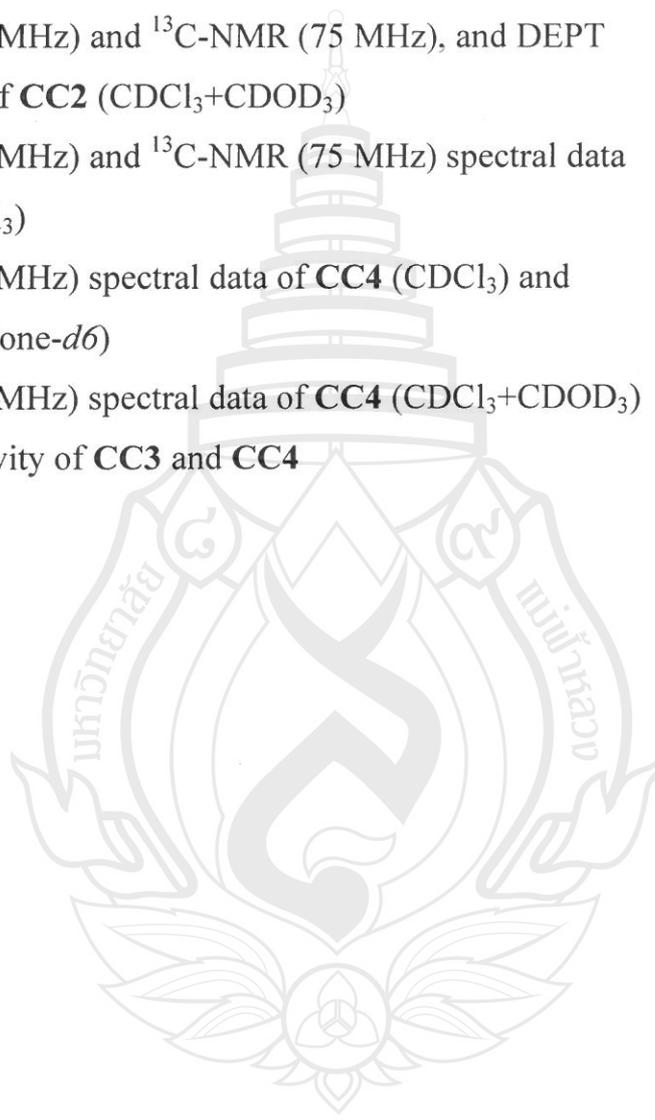
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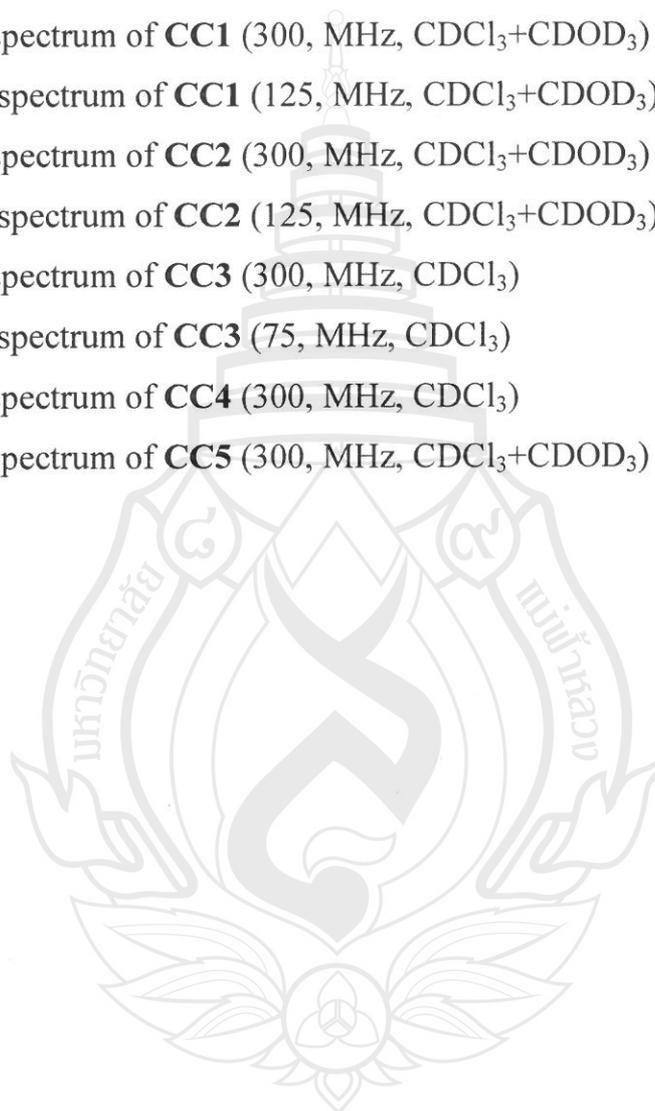
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ABBREVIATIONS AND SYMBOLS

<i>s</i>	=	<i>singlet</i>
<i>d</i>	=	<i>doublet</i>
<i>t</i>	=	<i>triplet</i>
<i>q</i>	=	<i>quartet</i>
<i>m</i>	=	<i>multiplet</i>
<i>dd</i>	=	<i>doublet of doublet</i>
<i>dt</i>	=	<i>doublet of triplet</i>
<i>br s</i>	=	<i>broad singlet</i>
<i>br m</i>	=	<i>broad multiplet</i>
<i>g</i>	=	gram
<i>nm</i>	=	nanometer
<i>m.p.</i>	=	melting point
cm^{-1}	=	reciprocal centimeter (wave number)
δ	=	chemical shift relative to TMS
<i>J</i>	=	coupling constant
$[\alpha]_D$	=	specific rotation
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ϵ	=	molar extinction coefficient
<i>m/z</i>	=	a value of mass divided by charge
$^{\circ}\text{C}$	=	degree Celsius
<i>MHz</i>	=	Mega Hertz
<i>ppm</i>	=	part per million
<i>c</i>	=	concentration
<i>IR</i>	=	Infrared

ABBREVIATIONS AND SYMBOLS (continued)

UV	=	Ultraviolet-Visible
MS	=	Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
COSY	=	Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
ROESY	=	Rotating from Overhauser Effect Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
TMS	=	tetramethylsilane
CDCl ₃	=	deuteriochloroform
CDOD ₃	=	deuteromethanol

CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

Synthesis of many important drugs makes use of natural product starting materials. Researches are conducted in order to find major constituents with biological activity to be used as drugs or in synthesis of analog or derivatives. Pure compounds extracted from many plants and many parts of the plants are explored and tested for biological activities. However, elucidation of chemical constituents from natural products and biological activity testing are only the initial step in the process of study to find new compounds and acquire basic knowledge of biological activity against fungi, malaria, AIDS, inflammation and cytotoxic activity. The important process is the application of the knowledge in pharmacology and medicine.

1.2 Objectives

The objectives of this project are involved:

1. To isolate and characterize compounds from the fruits of *C. cochinchinense*.
2. To test biological activity of pure compounds

1.3 Scope of study

1. Extraction and isolation of secondary metabolite from the fruits of *C. cochinchinense*.
2. Characterization of all isolates by spectroscopic methods, including UV, IR, NMR and MS.

1.4 Benefit

1. Some compounds, which were isolated the fruits of *C. cochinchinense*, might be showed significant biological activity.
2. Some active compounds might be applied into the related field i.e. pharmacy, cosmetics and agriculture.
3. Acquire basic knowledge of chemical compounds and biological activity.

4. This work might be published in international journals.

1.5 Review of Literatures

C. cochinchinense (Figure 1 A-C) belongs to Guttiferae family. The plant is distributed in several Southeast Asian countries. Among *Cartoxylum* six species are found in Thailand, *C. arborescens* (Vahl) Blume, *C. cochinchinense* (Lou.) Blume, *C. formosum* (Jack) Dyer, *C. formosum* (Jack) Dyer *spp. pruniflorum* (Kurz) Gogel, *C. maingayi* and *C. sumatranum* (Jack) Blume.

C. cochinchinense (Lour) Blume is shrub or trees, deciduous, 1.5-18 or 25 m tall, glabrous. The other characteristics of this plant are summarized below.

Trunks: is with tufted long spines on lower part.

Barks: is tray-yellow or gray-brown, smooth or finely striate.

Twigs: are somewhat compressed, glabrous and pink when young, interpetiolar scars not always continuous.

Petioles: are 2-3 mm, glabrous; leaf blades elliptic to oblong or lanceolate, 3-10.5 × 1-4 cm, papery, both surfaces glabrous, abaxially gray-green and with pellucid or dark glands, abaxially green, base obtuse to cuneate, apex abruptly acute or acuminate; midvein abaxially elevated, abaxially impressed; lateral veins 8-12 pairs, oblique, free; veins and veinlets reticulate, elevated on both surfaces.

Cymes: are axillary or extra-axillary and terminal, 1 or 2 or 3-flowered, pedunculate; peduncles 3-10 mm or longer.

Flowers: are 1-1.5 cm in diameter. Sepals are oblong, 5-7 × 2-5 mm, apex rounded, with dark linear glands on entire surface, accrescent. Petals are deep crimson to pink or pinkish yellow, obovate, 5-10 × 2.5-5 mm, with dark linear glands between veins, without a petal-scale, base cuneate, apex rounded. Stamen fascicles are 4-8 mm, stalk broad to slender. Fasciculates are oblong to obovate, cuculate, to 3 × 1-1.5 mm, apex thickened and recurved. Ovary conical is ca. 3 mm, glabrous; styles linear, ca. 2 mm, divaricate from base. Capsule is brown, ellipsoid, 0.8-1.2 mm, glabrous, to 2/3 covered by persistent calyx.

Seeds: 5 or 6-8 in each cell, obovoid, 6-8 × 2-3 mm.

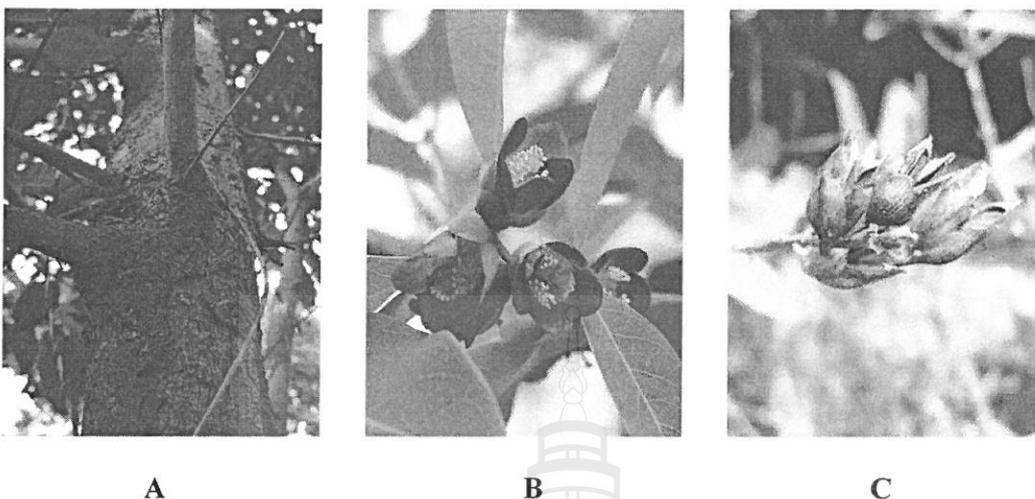


Figure 1 Stem bark, flowers and fruits of *C. cochinchinense* (A-C)

Plants in the *Cratoxylum* genus are well known to be rich source in a variety of compounds, for example xanthone, anthraquinone and flavonoid. The information from SciFinder scholar data based reveals several types of compounds, which present in the plants of *Cratoxylum* genus and can be classified into groups as follows: antharquinone, benzoic acid, carboxylic acid, coumarin, flavonoid, phenol, quinoid, tocotrienol, triterpene, visminol, xanthene and xanthone. These compounds are presented in Table 1.

Table 1 Compounds isolated from *Cratoxylum* genera

A = Antharquinone **B** = Benzoic acid **C** = Carboxylic acid
D = Flavonoid **E** = Phenol **F** = Tocotrienol
G = Triterpene **H** = Visminol **I** = Xanthene
J = Xanthone

Plant	Part	Compound	Bibliography
<i>C. arboresens</i>	Stem barks	3-Geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, A1	Pattanaprateeb, <i>et al.</i> , 2005
		1,7-Dihydroxyxanthone, J1	
		1,3-Dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone, J23	
		Fuscaxanthone C, J34	
<i>C. cochinchinense</i>	Barks	1,3,5,6-Tetrahydroxyxanthone, J3	Sia, <i>et al.</i> , 1995
		11-Hydroxy-1-isomangostin, J34	
		Xanthonolignoid, J41	
		Cratoxyxanthone, J46	
	Stem barks	3-Geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, A1	Ntuyen and Harrison, 1998
		Lupelo, G1	
		(13 <i>E</i> , 17 <i>E</i>)-Polypoda-7,13,17,21-tetraen-3 β -ol, G4	
		7-Geranyloxy-1,3-dihydroxyxanthone, J4	
		β -Mangostin, J16	
		2-Geranyl-1,3,7-trihydroxy-4-(3-methylbut-2-enyl)xanthone, J19	
1,3,7-Trihydroxy-2,4-di(3-methylbut-2-enyl)xanthone, J20			

Table 2 (Continued)

Plant	Part	Compound	Bibliography
<i>C. cochinchinense</i>	Trunk barks	δ -Tocotrienol, F1	Bennett, <i>et al.</i> , 1993
		δ -Tocotrienol dimmer, F2	
		5-(γ -Tocotrienyl)- γ -tocotrienol, F3	
		Friedelin, G2	
		Polypoda-8(26,13,17,21-tetraen-3 β -ol, G5	
		Mangostin, J15	
		β -Mangostin, J16	
		2-Geranyl-1,3,7-trihydroxy-4-(3,3-dimethylallyl)xanthone, J19	
		Cartoxylone, J21	
		Garcinone D, J22	
		Tovophyllin A, J45	
	Roots	β -Mangostin, J16	Laphookhieo, <i>et al.</i> , 2006
		α -Mangostin, J17	
		5- <i>O</i> -Methylcelebixanthone, J25	
		Celebixanthone, J26	
		Cochinchinone A, J30	
		1,3,7-Trihydroxy-2,4-di(3-methylbut-2-enyl)xanthone, J32	
Cochinchinone C, J40			

Table 1 (Continued)

Plant	Part	Compound	Bibliography
<i>C. formosum</i>	Roots	Astilbin, D1	Iinuma, <i>et al.</i> , 1996
		(-)-Epicatechin, D2	
		1,7-Dihydroxyxanthone (euxanthone), J1	
		1,7-Dihydroxy-8- dimethoxyxanthone, J5	
		2,7-Dihydroxy-1,8- dimethoxyxanthone, J6	
		1,7-Dihydroxy-4-methoxyxanthone, J7	
		1,4,7-Trihydroxyxanthone, J8	
		3,8-Dihydroxy-1,2- dimethoxyxanthone, J9	
		1,2,3,4,8-Pentamethoxyxanthone, J10	
		1,4,7-Trihydroxy-8- methoxyxanthone, J11	
		Machluraxanthone, J37	
	Leaves	Chlorogenic acid, C1	Maisuthisakul, <i>et al.</i> , 2007
		Dicaffeoylquinic acid, C2	
	Roots	3-Geranyloxy-6-methyl-1,8- dihydroxyanthraquinone, A1	Boonsri, <i>et al.</i> , 2006
		Vismiaquione, A2	
		Madagascin, A3	
		Gerontoxanthone I, J31	
		Formoxanthone A, J33	
		Formoxanthone C, J36	
Macluraxanthone, J38			

Table 1 (Continued)

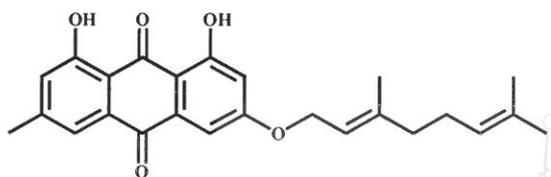
Plant	Part	Compound	Bibliography
<i>C. formosum</i>	Roots	Xanthone V ₁ , J39	Boonsri, <i>et al.</i> , 2006
		Formoxanthone B, J44	
<i>C. formosum</i> <i>ssp. pruniflorum</i>	Barks	3-Geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, A1	Boonnak, <i>et al.</i> , 2007
		Vismiaquinone, A2	
		11-Hydroxy-5-methoxy-2,2,9-trimethyl-2 <i>H</i> -antra(1,2-b)-pyran-7,12-dione, A4	
		Bianthrone J, B1	
		Bivismiaquinone, B2	
		Biantrone A ₁ , B3	
		Vismones D, H2	
		Vismones E, H3	
<i>C. maingayi</i>	Woods	1,7-Dihydroxyxanthone (euxanthone), J1	Pinto, <i>et al.</i> , 1997
		1,5-Dihydroxy-8-methoxyxanthone, J12	
		5-Hydroxy-2,3,4,8-tetramethoxyxanthone, J14	
		7-Hydroxy-1,2,3,8-tetramethoxyxanthone, J2	Kijjoa, <i>et al.</i> , 1998
		1,7-Dihydroxy-8-methoxyxanthone, J5	
		1,7-Dihydroxy-4-methoxyxanthone, J21	
<i>C. pruniflorum</i>	Leaves	(-)-Epicatechin, D2	Van, <i>et al.</i> , 1988
		Hyperoside, D4	
		Mangiferin, J43	

Table 1 (Continued)

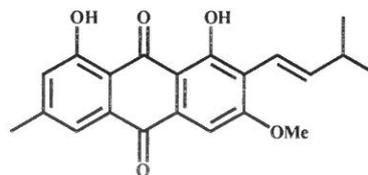
Plant	Part	Compound	Bibliography
<i>C. pruniflorum</i>	Leaves	Quercetin, D3	Kitanov, <i>et al.</i> , 1998
		Hyperoside, D4	
		6,7-Dihydro-1H-xanthen-2(3H)-one, I1	
		Isomagiferin, J42	
		Mangiferin, J43	
	Stems	(-)-Epicatechin, D2	Dan The Van, <i>et al.</i> , 1998
		Hyperoside, D4	
		Isomangiferin, J42	
		Mangiferin, J53	
	Not specified	Norathyriol, J24	Bennett and Lee, 1989
		Isomangiferin, J42	
		Mangiferin, J53	
<i>C. prunifolium</i>	Leaves	Epicatechin 3- <i>O</i> -gallate, E1	Cao, <i>et al.</i> , 2000
		Epigallocatechin 3- <i>O</i> -gallate, E2	
<i>C. sumatranum</i>	Leaves	Cratoxyarborenone A, J27	Seo, <i>et al.</i> , 2002
		Cratoxyarborenone B, J28	
		Cratoxyarborenone E, J29	
		Cratoxyarborenone D, J35	
	Stem Barks	Cratoxyarborequinone A, A5	
		Cratoxyarborequinone B, A6	
		2,4,6-Trihydroxybenzophenone 4- <i>O</i> -geranyl ether, E3	
		δ -Tocotrienol, F1	
		Betulinic acid, G3	
		Vismione B, H1	
		Cratoxyarborenone C, J18	
	Twigs	Cratoxyarborenone F, J13	

Structures

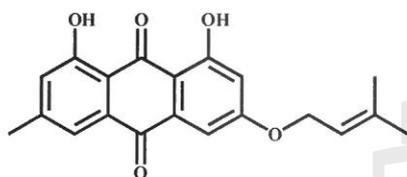
A = Anthraquinone



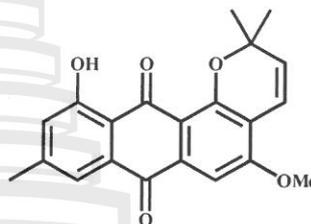
3-Geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, **A1**



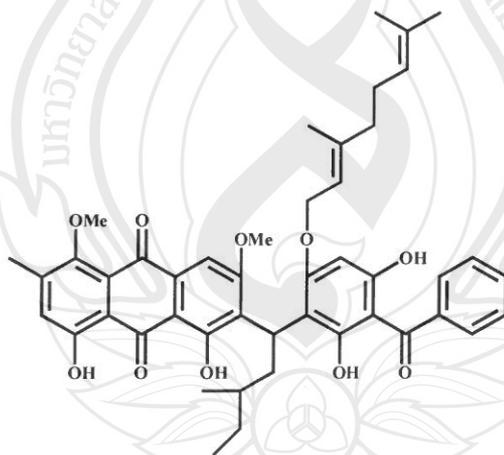
Vismiaquione, **A2**



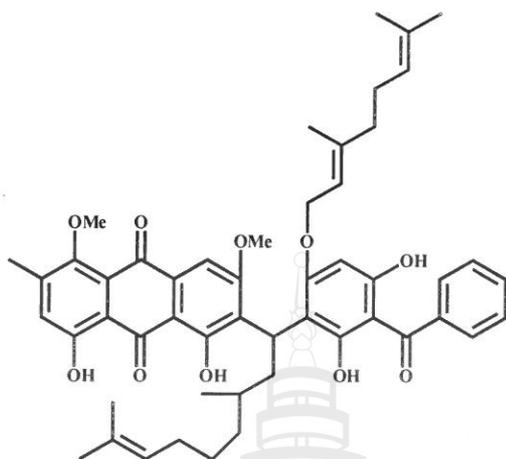
Madagascin, **A3**



11-Hydroxy-5-methoxy-2,2,9-trimethyl-2H-antra(1,2-b)-pyran-7,12-dione, **A4**

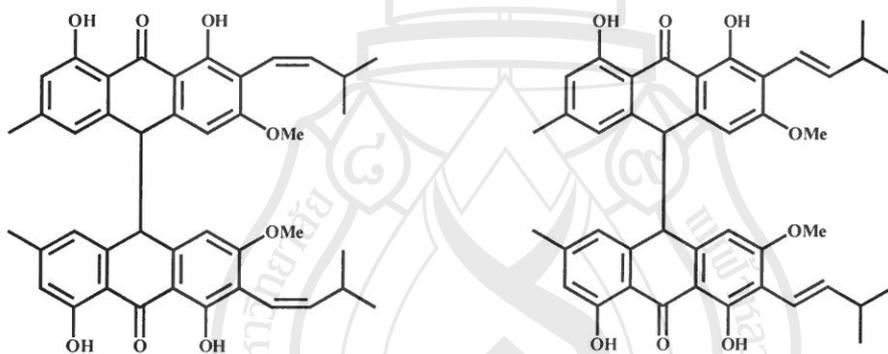


Cratoxyarborequinone A, **A5**



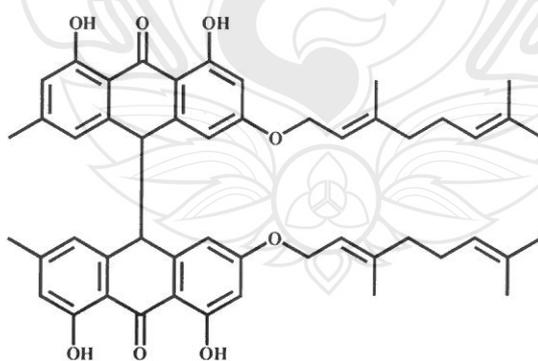
Cratoxyarborequinone B, A6

B = Biquinones



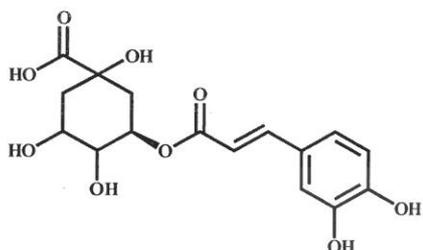
Bianthrone J, B1

Bivismiaquinone, B2

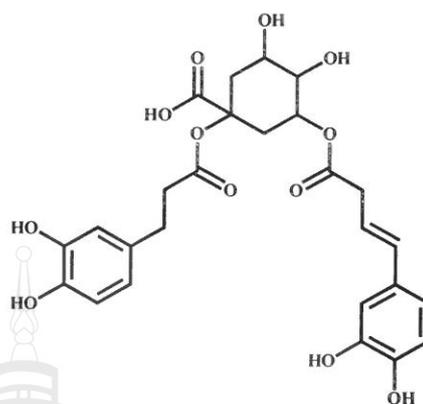


Bianthrone A₁, B3

C = Carboxylic acid



Chlorogenic acid, C1



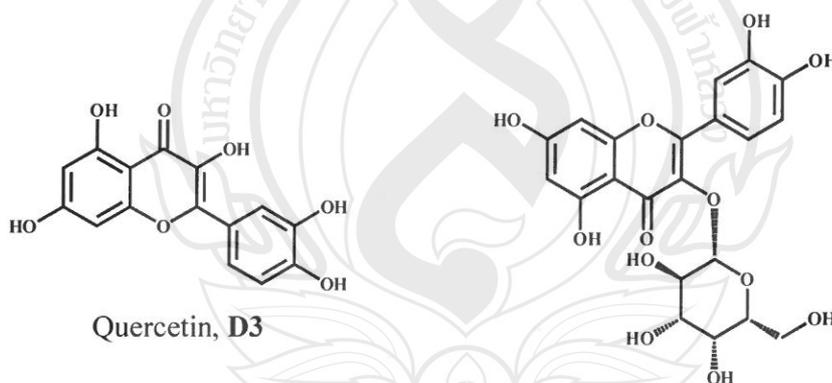
Dicafeoylquinic acid, C2

D = Flavonoids



Astilbin, D1

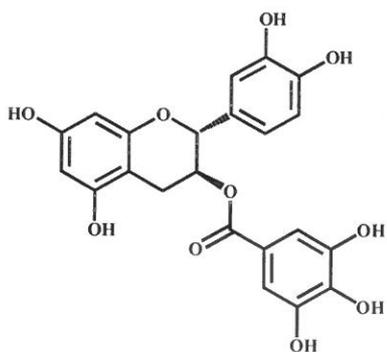
(-)-Epicatechin, D2



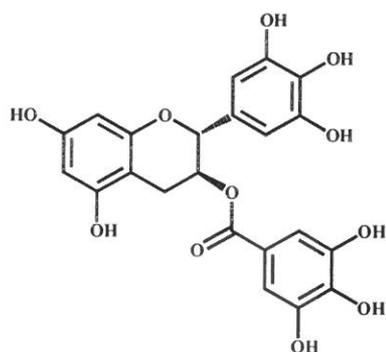
Quercetin, D3

Hyperoside, D4

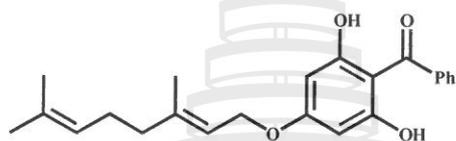
E = Phenol



Epicatechin 3-*O*-gallate, **E1**

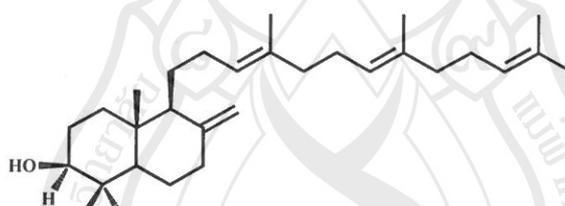


Epigallocatechin 3-*O*-gallate, **E2**

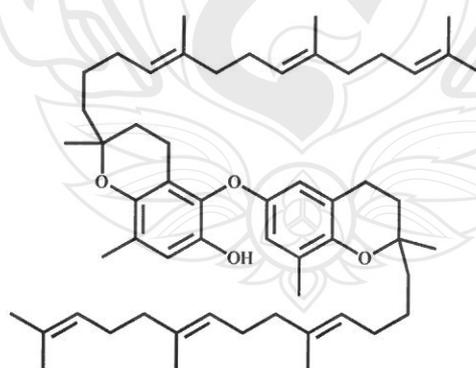


2,4,6-Trihydroxybenzophenone 4-*O*-geranyl ether, **E3**

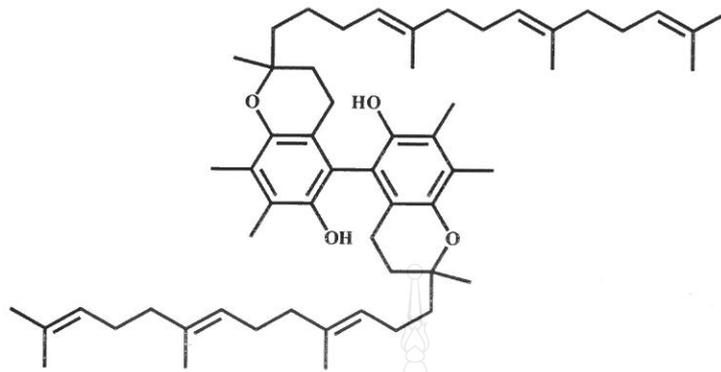
F = Tocotrienol



δ -Tocotrienol, **F1**

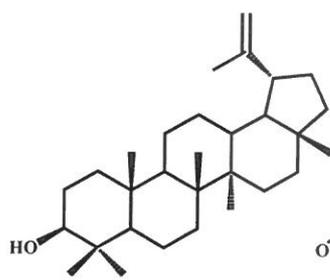


δ -Tocotrienol dimer, **F2**

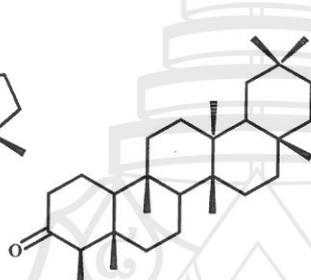


5-(γ -Tocotrienyl)- γ -tocotrienol, **F3**

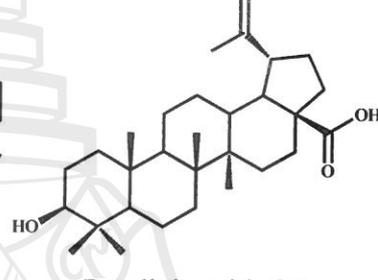
G = Triterpene



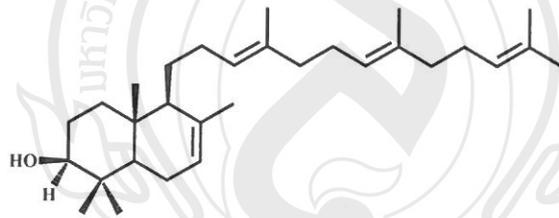
Lupelo, **G1**



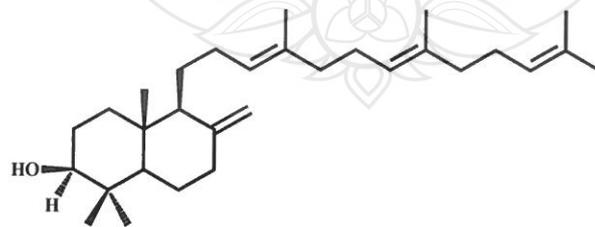
Friedelin, **G2**



Betulinic acid, **G3**

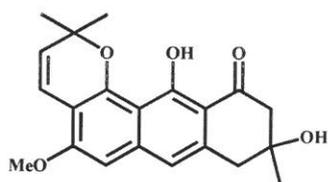


(13*E*, 17*E*)-Polyoda-7,13,17,21-tetraen-3 β -ol, **G4**

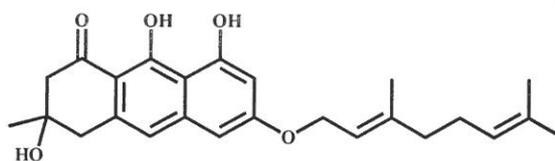


Polyoda-8(26,13,17,21-tetraen-3 β -ol, **G5**

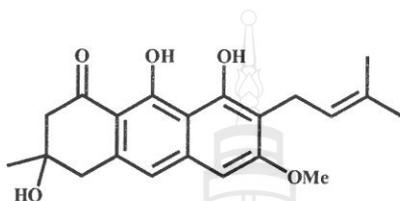
H = Visminol



Vismione B, **H1**

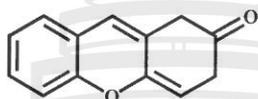


Vismione D, **H2**



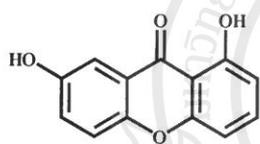
Vismione E, **H3**

I = Xanthene



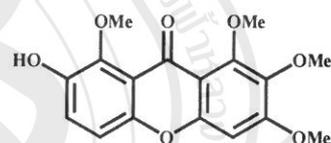
6,7-Dihydro-1H-xanthen-2(3H)-one, **I1**

J = Xanthone

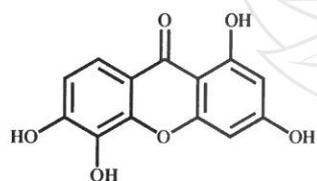


1,7-Dihydroxyxanthone (euxanthone),

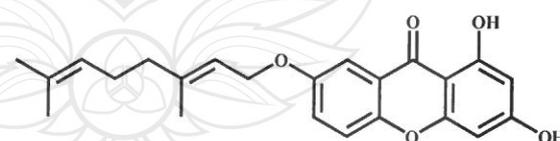
J1



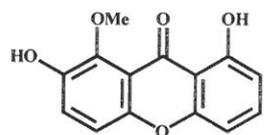
7-Hydroxy-1,2,3,8-tetramethoxyxanthone, **J2**



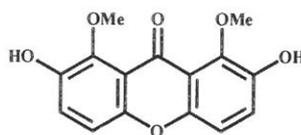
1,3,5,6-Tetrahydroxyxanthone, **J3**



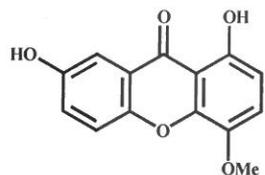
7-Geranyloxy-1,3-dihydroxyxanthone, **J4**



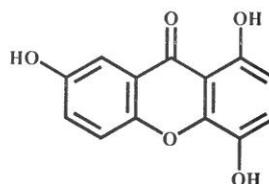
1,7-Dihydroxy-8-methoxyxanthone, **J5**



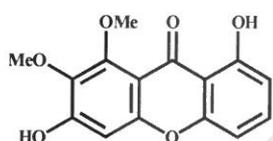
2,7-Dihydroxy-1,8-dimethoxyxanthone, **J6**



1,7-Dihydroxy-4-methoxyxanthone, **J7**

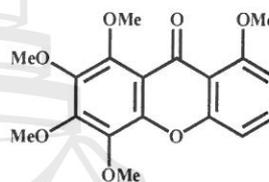


1,4,7-Trihydroxyxanthone, **J8**

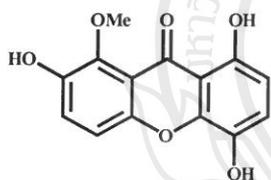


3,8-Dihydroxy-1,2-dimethoxyxanthone,

J9

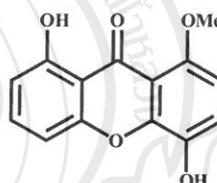


1,2,3,4,8-Pentamethoxyxanthone, **J10**



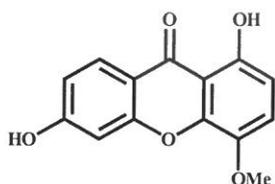
1,4,7-Trihydroxy-8-methoxyxanthone,

J11

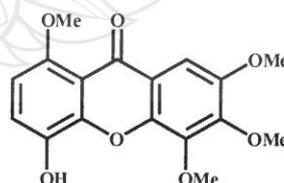


1,5-Dihydroxy-8-methoxyxanthone,

J12

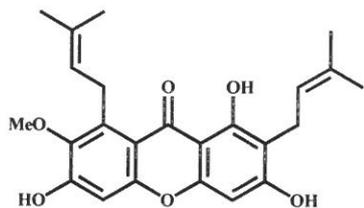


Cratoxyarborenone F, **J13**

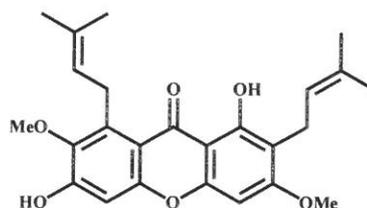


5-Hydroxy-2,3,4,8-tetramethoxyxanthone,

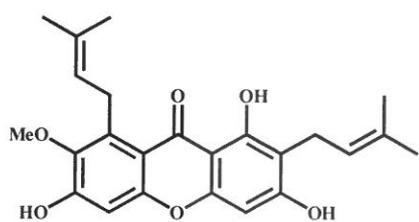
J14



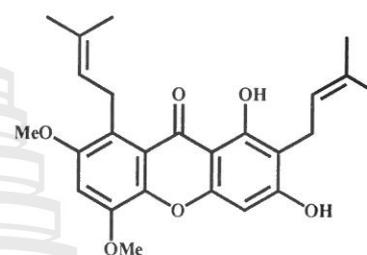
Mangostin, **J15**



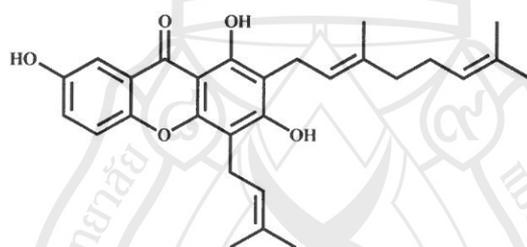
β -Mangostin, **J16**



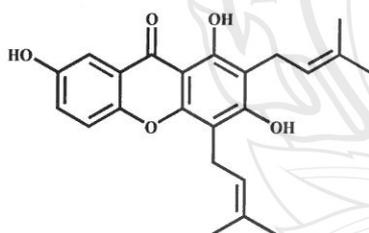
α -Mangostin, **J17**



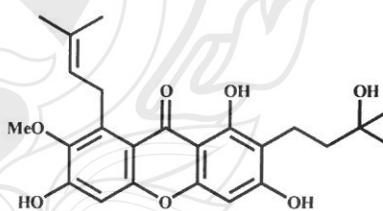
Cratoxyarborenone C, **J18**



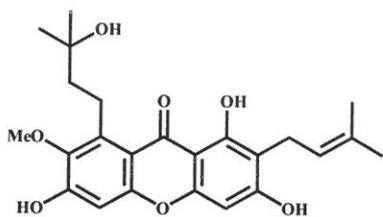
2-Geranyl-1,3,7-trihydroxy-4-(3-methylbut-2-enyl)xanthone, **J19**



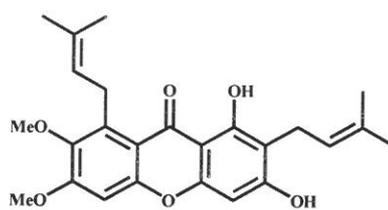
1,3,7-Trihydroxy-2,4-di(3-methylbut-2-enyl)xanthone, **J20**



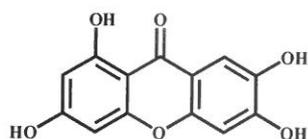
Cratoxylone, **J21**



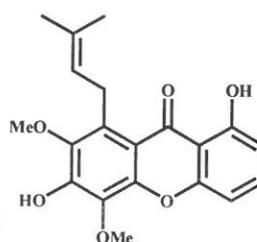
Garcinone D, **J22**



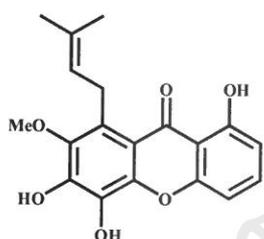
1,3-Dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone, **J23**



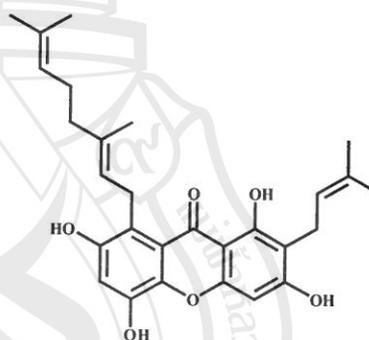
Norathyriol, **J24**



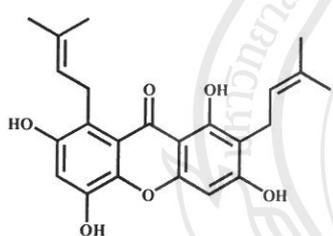
5-O-Methylcelebixanthone, **J25**



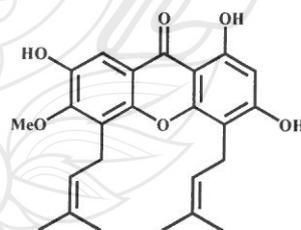
Celebixanthone, **J26**



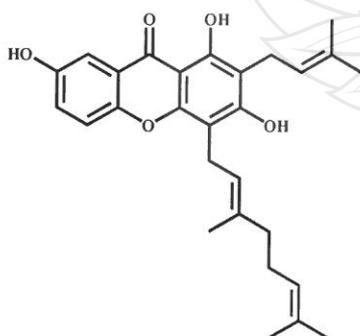
Cratoxyarborenone A, **J27**



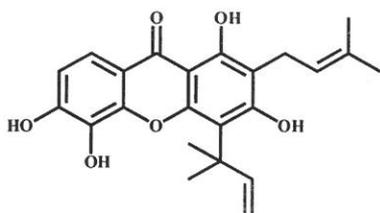
Cratoxyarborenone B, **J28**



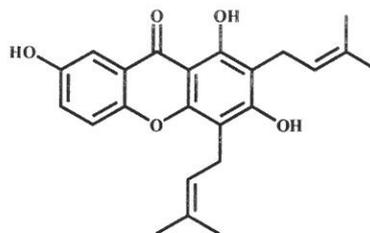
Cratoxyarborenone E, **J29**



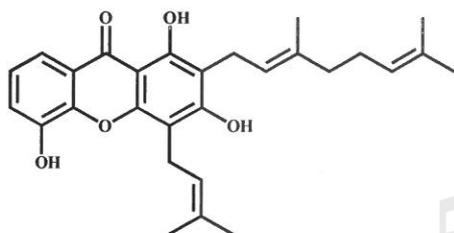
Cochinchinone A, **J30**



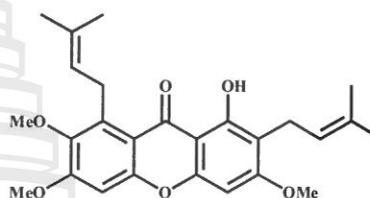
Gerontoxanthone I, **J31**



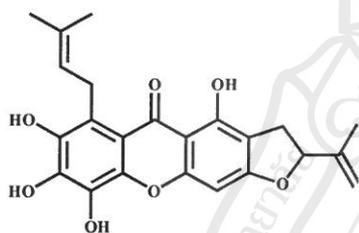
1,3,7-Trihydroxy-2,4-di(3-methylbut-2-enyl)xanthone, **J32**



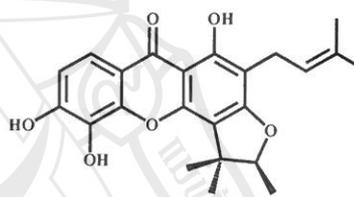
Formoxanthone A, **J33**



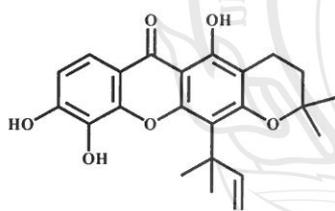
Fuscaxanthone C, **J34**



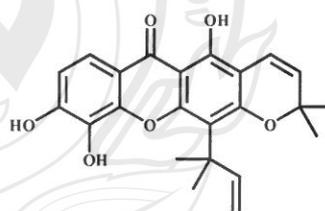
Cratoxyarborenone D, **J35**



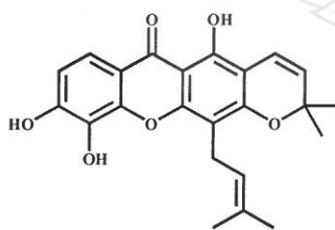
Formoxanthone C, **J36**



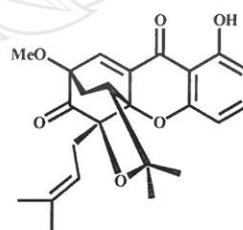
Machluraxanthone, **J37**



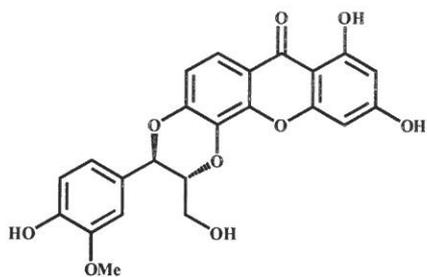
Macluraxanthone, **J38**



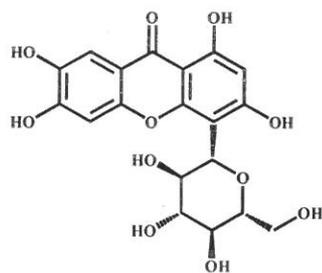
Xanthone V₁, **J39**



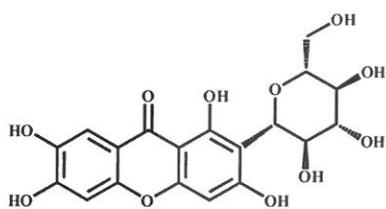
Cochinchinone C, **J40**



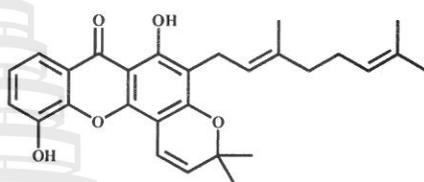
Xanthonolignoid, J41



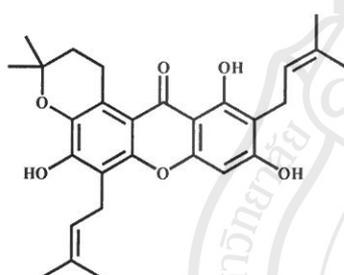
Isomagiferin, J42



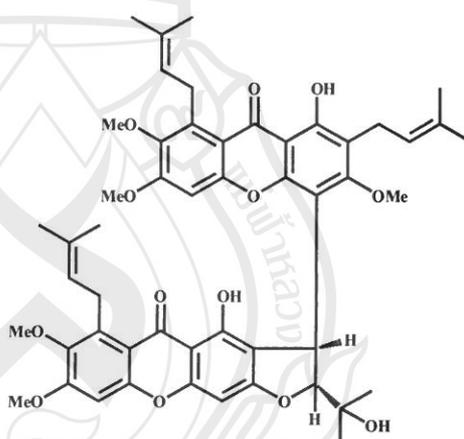
Mangiferin, J43



Formoxanthone B, J44



Tovophyllin A, J45



Cratoxyxanthone, J46

According to no phytochemical examination on the fruits *C. cochinchinense* in the SciFinder scholar data base, this prompted us to investigate their chemical constituents in order to provide additional information of the plants. The objectives of this project are to isolate, purify and structure elucidate compounds, isolated from the fruits *C. cochinchinense*.



CHAPTER 2

MATERIAL AND METHODS

2.1 Instruments and Chemicals

Melting points were determined using a Fisher-John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. UV spectra were measured with UV-160A spectrophotometers (Shimadzu). The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded using 400 and/ or 300 MHz Bruker FTNMR Ultra Shield spectrometers. Chemical shifts were recorded in parts per million (δ) in CDCl_3 and/or CD_3OD with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F₂₅₄ (Merck, 230-400 Mesh ASTM) and silica gel 100 (Merck, 70-230 Mesh ASTM), respectively. Precoated plates of silica gel 60 F₂₅₄ were used for analytical purposes.

2.2 Plant materials

The fruits of *C. cochinchinense* were also collected from our campus in December 2006. Botanical identification was achieved through comparison with a voucher specimen No. SL-1 (PSU) in the herbarium collection of Department of Biology, Prince of Songkla University, Songkhla, Thailand.

2.3 Biological Assays

Anti-malaria assay

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K₁, multidrug resistant), using the method of Trager and Jensen (1976). Quantitative assessment of *in vitro* malarial activity was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al. (1979). The inhibitory concentration (IC₅₀) represented the concentration that caused 50% reduction in parasite growth which was indicated by the *in vitro* uptake of

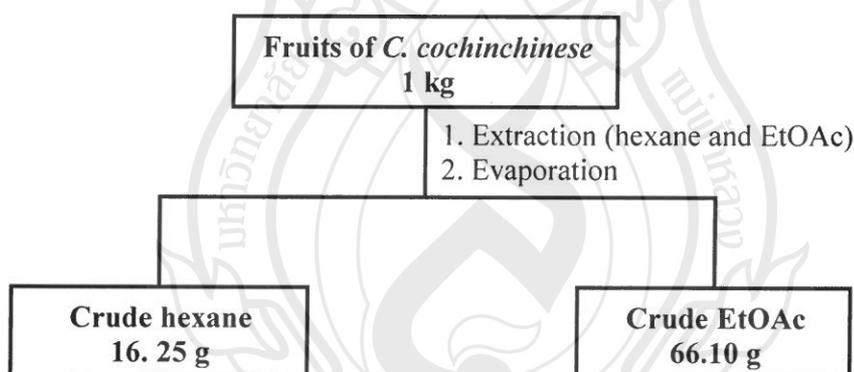
[³H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (IC₅₀ 0.00137 µg/ml).

Cytotoxic assay

The procedures for cytotoxic assay were performed by sulphorhodamine B (SRB) assay (anti-KB and MCF-7) and colorimetric method (anti-NCI-H187) as described by Skehan et al. (1990). In this study, three cancer cell lines, MCF-7 (breast cancer), NCI-H187 (human, small cell lung cancer) and KB (oral human epidermal carcinoma) were used. Ellipticin and doxorubicin were the reference substances in this study and the IC₅₀ values are summarized in Table 7.

2.4 Extraction

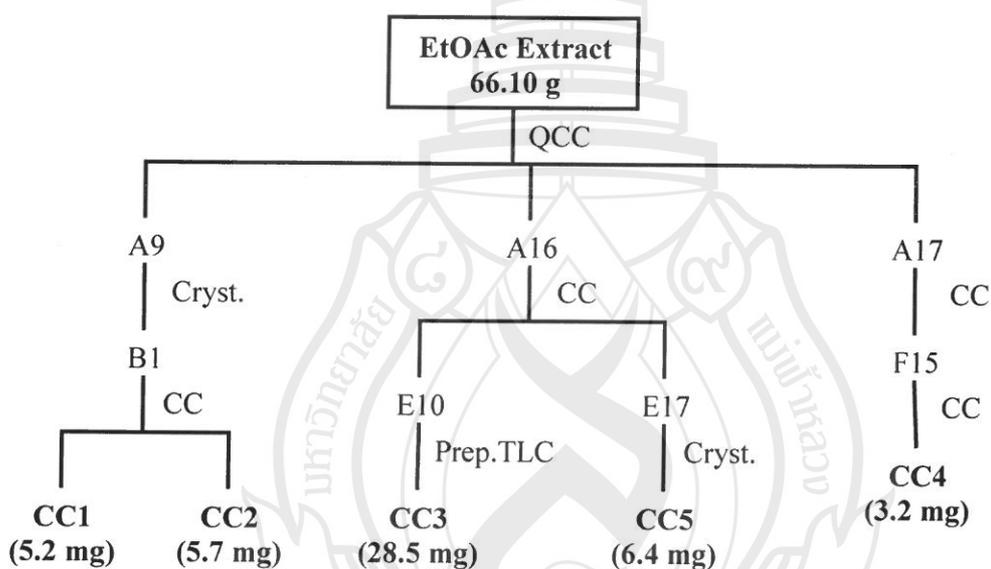
The fruits of *C. cochinchinense* (1 kg.) were extracted with hexane and EtOAc, respectively, over the period of 3 days each at room temperature and evaporated under reduced pressure to afford crude hexane (16.25 g) and EtOAc extracts (66.10 g).



2.5 Isolation

The EtOAc extract (66.25 g) of the fruits of *C. cochinchinense* was subjected to QCC over silica gel and eluted with gradient of EtOAc-acetone to afford 20 fractions (A1-A20). Fraction A9 upon evaporating under reduced pressure gave yellow solid, which washed with hexane and DCM to afford fraction B1. This fraction (352.7 mg)

was subjected to repeated CC with 100% DCM to yield compound **CC1** (5.2 mg) and **CC2** (5.7 mg). Fraction A16 (555.5 g) was purified by CC with gradient mixtures of 5-20% acetone-hexane to afford 19 subfractions (E1-E19). Subfraction E10 (43.5 mg) was further purified by prep. TLC with 15% acetone-hexane to give **CC3** (28.5 mg). Fraction E17 upon evaporating under reduced pressure afforded solid, which was further washed with hexane and DCM to give compound **CC5** (6.4 mg). Fraction A17 (3.1461 g) was further purified by CC with 20% acetone-hexane to afford 20 subfractions (F1-F20). Subfraction F15 (89.3 mg) was repeated CC using 2.5% acetone-chloroform to give compound **CC4** (3.2 mg). The summary of isolations of compounds from *C. cochinchinense* was showed in scheme 5.



Scheme 5 Isolation of **CC1-CC5**

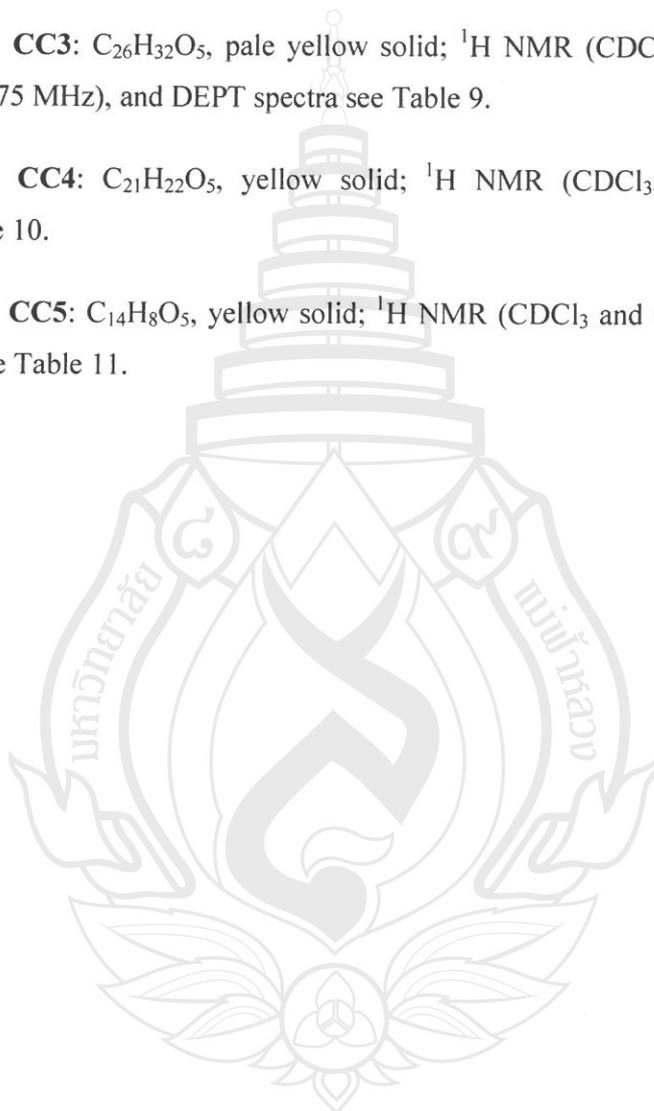
Compound CC1: $C_{23}H_{24}O_6$, pale yellow solid, m.p. 160–162 °C $[\alpha]_D^{25} +112.6^\circ$ (c 0.025, MeOH). UV λ_{max}^{MeOH} nm (log ϵ): 368 (3.88), 309 (4.14), 258 (4.53), 235 (4.51), 204 (4.30). IR (neat) ν_{max} : 3436, 1646 cm^{-1} . For 1H NMR ($CDCl_3$ and CD_3OD , 300 MHz), ^{13}C NMR ($CDCl_3+CD_3OD$, 75 MHz), and DEPT spectra, see Table 7. HREIMS m/z 396.3709 $[M]^+$.

Compound CC2: $C_{23}H_{24}O_6$, pale yellow solid, m.p. 154–155 °C $[\alpha]_D^{25} +116.6^\circ$ (*c* 0.03, MeOH). UV λ_{max}^{MeOH} nm (log ϵ): 374 (3.67), 307 (4.06), 258 (4.42), 235 (4.32), 203 (4.20). IR (neat) ν_{max} : 3435, 1638 cm^{-1} . For 1H NMR ($CDCl_3$ and CD_3OD , 300 MHz), ^{13}C NMR ($CDCl_3+CD_3OD$, 75 MHz), and DEPT spectra, see Table 8. HREIMS m/z 396.3709 $[M]^+$.

Compound CC3: $C_{26}H_{32}O_5$, pale yellow solid; 1H NMR ($CDCl_3$, 300 MHz), ^{13}C NMR ($CDCl_3$, 75 MHz), and DEPT spectra see Table 9.

Compound CC4: $C_{21}H_{22}O_5$, yellow solid; 1H NMR ($CDCl_3$, 300 MHz) spectrum, see Table 10.

Compound CC5: $C_{14}H_8O_5$, yellow solid; 1H NMR ($CDCl_3$ and CD_3OD , 300 MHz) spectrum, see Table 11.



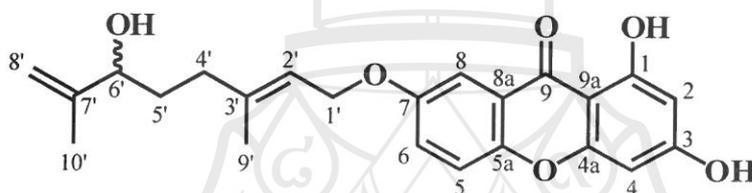
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Structural elucidation of compounds isolated from the fruits of *C. cochinchinense*

Purification of the EtOAc extract of the fruits of *C. cochinchinense* by chromatographic techniques yielded five phenolic compounds, **CC1** (5.2 mg), **CC2** (5.7 mg), **CC3** (28.5 mg), **CC4** (3.2 mg) and **CC5** (6.4 mg). Two of them are new compounds (**CC1** and **CC2**). Their structures were determined using 1D and 2D NMR spectroscopic data.

3.2.1. Compound CC1



Compound **CC1** was isolated as pale yellow solid with a molecular formula of $C_{23}H_{24}O_6$ based on the $[M]^+$ ion at m/z 396.3709 in the HREIMS. The IR spectrum exhibited absorption bands in the range of 1646 (carbonyl) and 3436 cm^{-1} (hydroxyl). The ^{13}C NMR and DEPT spectra (Table 2) revealed 23 carbons, including 2 methyls (δ 15.6 and 17.0), 4 methylenes (δ 35.3, 32.7, 65.2 and 110.4), 7 methines (δ 74.7, 93.7, 97.7, 106.3, 118.8, 124.9 and 119.4) and 10 quaternary carbons (δ 102.4, 120.4, 141.3, 147.2, 150.6, 155.2, 158.1, 163.1, 165.9 and 180.3). The ^1H NMR spectral data (Table 2) showed a downfield hydroxyl proton resonance at δ 13.00 (1-OH) together with two sets of aromatic protons. The first set displayed two *meta*-coupled protons appeared at δ 6.32 (1H, *d*, $J = 1.8$ Hz, H-4) and 6.19 (1H, *d*, $J = 1.8$ Hz, H-2). The other set is a typical pattern of 1,2,4-trisubstituted benzene ring appeared at δ 7.39 (1H, *d*, $J = 9.3$ Hz, H-5), 7.33 (1H, *dd*, $J = 9.3$ and 2.7 Hz, H-6) and 7.55 (1H, *d*, $J = 2.7$ Hz, H-8). With the combination of COSY, HMQC and HMBC spectra, a 6-hydroxy-3,7-

dimethylocta-2,7-dienyloxy moiety (*O*-geranyl moiety) was evident from ¹H NMR signals at δ 5.48 (1H, *t*, J = 6.6 Hz, H-2'), 4.75 (1H, *s*, H-8') 4.96 (1H, *s*, H-8'), 4.65 (2H, *d*, J = 6.6 Hz, H-1'), 3.98 (1H, *t*, J = 6.6 Hz, H-6'), 2.13 (2H, *m*, H-4'), 1.82 (3H, *s*, H-9'), 1.70 (3H, *s*, H-10') and 1.63 (2H, *m*, H-5'). This moiety was supported by the molecular ion peak at m/z 243 ([M-C₁₀H₁₇O]⁺) in HREIMS spectrum. This moiety was placed at C-7 due to the correlation of H-1' (δ 4.65), H-8 (δ 7.55) and (δ 7.33) to C-7 (δ 155.2) in the HMBC spectral data (Figure 2). The interaction between H-1' (δ 4.65) and H-8 (δ 7.55) in NOSEY spectrum data (Figure 2) was also confirmed the location of *O*-geranyl moiety. Therefore, compound **CC1** was identified as 1,3-dihydroxy-7-(6-hydroxy-3,7-dimethylocta-2,7-dienyloxy)-xanthen-9-one (cochinxanthone A).

Table 2 ¹H-NMR (300 MHz), ¹³C-NMR (75 MHz) and DEPT spectral data of **CC1** in CDCl₃ and CD₃OD

Position	δ_C	δ_H (mult., J in Hz)	DEPT
1	163.1	–	C
2	97.7	6.19 (<i>d</i> , J = 1.8)	CH
3	165.9	–	C
4	93.7	6.32 (<i>d</i> , J = 1.8)	CH
4a	158.1	–	C
5a	150.6	–	C
5	118.8	7.39 (<i>d</i> , J = 9.3)	CH
6	124.9	7.33 (<i>dd</i> , J = 9.3, 2.7)	CH
7	155.2	–	C
8	106.3	7.55 (<i>d</i> , J = 2.7)	CH
8a	120.4	–	C
9	180.3	–	C
9a	102.4	–	C
1'	65.2	4.65 (<i>d</i> , J = 6.6)	CH ₂
2'	119.4	5.48 (<i>t</i> , J = 6.6)	CH
3'	141.3	–	C

Table 2 (Continued)

Position	δ_C	δ_H (mult., J in Hz)	DEPT
4'	35.3	2.13 (<i>m</i>)	CH ₂
5'	32.7	1.63 (<i>m</i>)	CH ₂
6'	74.7	3.98 (<i>t</i> , $J = 6.6$)	CH
7'	147.2	–	C
8'	110.4	4.75 (<i>s</i>); 4.96 (<i>s</i>)	CH ₂
9'	15.6	1.82 (<i>s</i>)	CH ₃
10'	17.0	1.70 (<i>s</i>)	CH ₃
1-OH	–	13.00 (<i>s</i>)	–

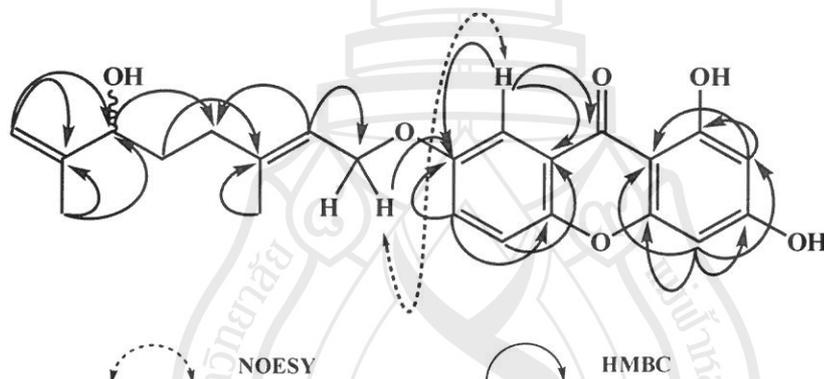
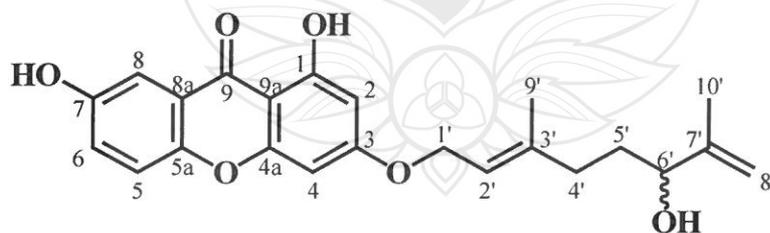


Figure 7 HMBC and NOESY correlations of **CC1**

3.2 Compound CC2



Compound **CC2** was isolated as a pale yellow solid. This compound had the same molecular formula as **CC1**, C₂₃H₂₄O₆ (m/z 396.3748 [M^+]). The ¹H and ¹³C NMR spectrum of **CC2** (Table 3) were close to that of **CC1**, except the chemical shift

values of aromatic and chelated hydroxyl protons were different (Table 3). In addition, the R_f value on the TLC of **CC2** ($R_f = 0.33$, 2.5% acetone-DCM) was also different from **CC1** ($R_f = 0.40$, 2.5% acetone-DCM). These results implied that the *O*-geranyl side chain of **CC2** was located at C-3. Finally, the HMBC correlations also supported the structure. Therefore, cochinxanhone B was identified as 1,7-dihydroxy-3-(6-hydroxy-3,7-dimethylocta-2,7-diexyloxy)-xanthone-9-one, the structural isomer of **CC1**.

Table 3 $^1\text{H-NMR}$ (300 MHz), $^{13}\text{C-NMR}$ (75 MHz) and DEPT spectral data of **CC2** in CDCl_3 and CD_3OD

Position	δ_{C}	δ_{H} (mult., J in Hz)	DEPT
1	162.8	–	C
2	97.1	6.28 (<i>br s</i>)	CH
3	166.1	–	C
4	92.7	6.32 (<i>br s</i>)	CH
4a	157.9	–	C
5a	149.9	–	C
5	118.6	7.37 (<i>d</i> , $J = 8.7$)	CH
6	124.1	7.26 (<i>dd</i> , $J = 8.7, 2.4$)	CH
7	153.9	–	C
8	108.1	7.59 (<i>d</i> , $J = 2.4$)	CH
8a	120.8	–	C
9	180.6	–	C
9a	103.0	–	C
1'	65.2	4.62 (<i>d</i> , $J = 6.6$)	CH ₂
2'	118.8	5.47 (<i>t</i> , $J = 6.3$)	CH
3'	141.7	–	C
4'	35.2	2.10 (<i>m</i>)	CH ₂
5'	32.7	1.64 (<i>m</i>)	CH ₂
6'	74.6	4.00 (<i>t</i> , $J = 6.3$)	CH
7'	147.2	–	C

Table 3 (Continued)

Position	δ_C	δ_H (mult., J in Hz)	DEPT
8'	110.3	4.86 (<i>s</i>); 4.95 (<i>s</i>)	CH ₂
9'	15.5	1.82 (<i>s</i>)	CH ₃
10'	16.3	1.71 (<i>s</i>)	CH ₃
1-OH	–	12.80 (<i>s</i>)	–

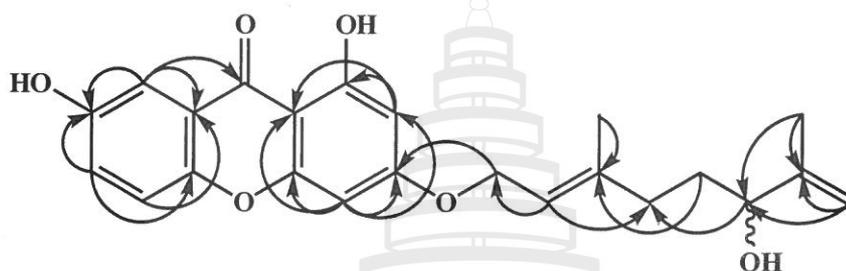
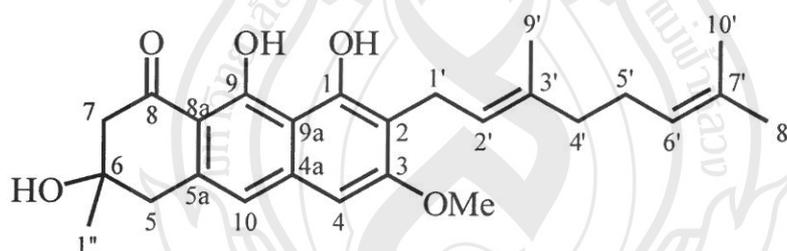


Figure 3 HMBC correlations of CC2

3.3 Compound CC3



Compound CC3 (C₂₆H₃₂O₅) was isolated as pale yellow solid. The ¹³C NMR and DEPT spectral data (Table 4) revealed 26 carbons, including 5 methyls (δ 16.1, 17.6, 25.6, 28.8 and 55.5), 5 methylenes (δ 21.9, 26.7, 39.8, 43.3 and 51.0), 4 methines (δ 97.7, 117.5, 122.1 and 124.4) and 12 quaternary carbons (δ 71.0, 108.0, 108.0, 114.8, 131.1, 134.0, 135.5, 138.9, 156.0, 162.0, 165.8 and 201.5). The ¹H NMR spectral data (Table 4) showed a downfield hydroxyl proton at 9.44 (*s*, 9-OH) together with a methoxyl moiety at δ 3.84 (3H, *s*, 3-OCH₃). In addition, ¹H NMR also displayed geranyl moiety at δ 3.44 (2H, *d*, J = 6.9, H-1'), 5.23 (1H, *m*, H-2'), 1.93-2.06 (2H, *m*, H-4'), 1.93-2.06 (2H, *m*, H-5'), 5.06 (1H, *m*, H-6'), 1.56 (3H, *s*, H-8'),

1.63 (3H, *s*, H-9') and 1.63 (3H, *s*, H-10'). Comparison the ¹H NMR of CC3 with vismione F, both compounds showed almost identical ¹H NMR spectrum. Therefore, CC3 should be identified as vismione F (Cassinelli and Geroni, 1986.).

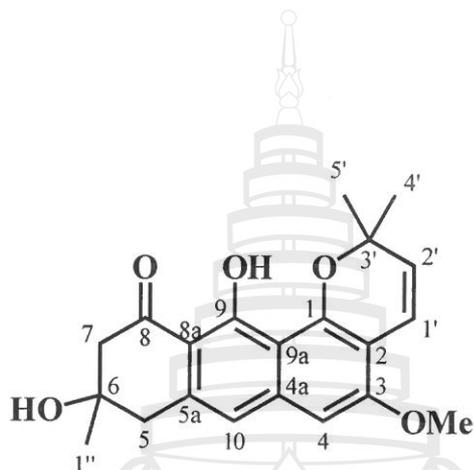
Table 4 ¹H-NMR (300 MHz), ¹³C-NMR (75 MHz) and DEPT spectral data of CC3 in CDCl₃

Position	δ _C	δ _H (mult., <i>J</i> in Hz)	DEPT
1	156.0	–	C
2	114.8	–	C
3	165.8	–	C
4	124.4	6.84 (<i>s</i>)	CH
4a	108.0	–	C
5a	108.0	–	C
5	39.8	2.96 (<i>s</i>)	CH ₂
6	71.0	–	C
7	51.0	2.74 (<i>s</i>)	CH ₂
8	201.5	–	C
8a	134.0	–	C
9	162.0	–	C
9a	131.1	–	C
10	97.7	6.52 (<i>s</i>)	CH
1'	21.9	3.44 (<i>d</i> , <i>J</i> = 6.9)	CH ₂
2'	122.1	5.23 (<i>m</i>)	CH
3'	135.5	–	C
4'	43.3	1.93-2.06 (<i>m</i>)	CH ₂
5'	26.7	1.93-2.06 (<i>m</i>)	CH ₂
6'	117.5	5.06 (<i>m</i>)	CH
7'	138.9	–	C
8'	17.6	1.63 (<i>s</i>)	CH ₃
9'	16.1	1.56 (<i>s</i>)	CH ₃
10'	25.6	1.63 (<i>s</i>)	CH ₃

Table 4 (Continued)

Position	δ_C	δ_H (mult., J in Hz)	DEPT
1''	28.8	1.43 (<i>s</i>)	CH ₃
3-OCH ₃	55.5	1.63 (<i>s</i>)	CH ₃
9-OH	–	9.44 (<i>s</i>)	–

3.4 Compound CC4



Compound **CC4** (C₂₁H₂₂O₅) was isolated as yellow solid. The ¹H NMR spectrum of **CC4** (Table 5) was close to those of **CC3**, expect that at C-2 was showed the ¹H NMR signal of chromene ring at δ 6.62 (1H, *d*, J = 9.9 Hz, H-1'), 5.52 (1H, *d*, J = 9.9 Hz, H-2'), 1.45 (3H, *s*, H-4') and 1.48 (3H, *s*, H-5'). Therefore, vismione B was identified to be **CC4** (Botta, *et al.*, 1982 and Seo, *et al.*, 2002).

Table 5 ¹H-NMR (300 MHz) spectral data of **CC4** in CDCl₃ and vismione B in Acetone-d₆

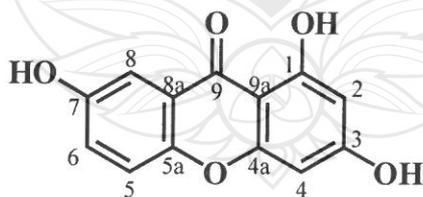
Position	δ_H (mult., J in Hz)	
	CC4	Vismione B*
1	–	–
2	–	–
3	–	–
4	6.71 (<i>s</i>)	6.73
4a	–	–
5a	–	–

Table 5 (Continued)

Position	δ_H (mult., J in Hz)	
	CC4	Vismione B*
5	2.96 (s)	3.00
6	—	—
7	2.74 (s)	2.80
8	—	—
8a	—	—
9	—	—
9a	—	—
10	6.40 (s)	6.83
1'	6.62 (d, $J=9.9$)	—
2'	5.52 (d, $J=9.9$)	—
3'	—	—
4'	1.45 (s)	—
5'	1.48 (s)	—
1''	1.34 (s)	—
3-OCH ₃	3.84 (s)	3.93
9-OH	14.69 (s)	14.70

* Botta, *et al.*, 1982

3.5 Compound CC5



Compound **CC5** (C₁₄H₈O₅) was isolated as pale yellow solid. The ¹H NMR spectral data (Table 6) showed a downfield hydroxyl proton at δ 12.92 (1-OH) together with two sets of aromatic protons, including two *meta*-coupled protons at δ

6.25 (1H, *d*, *J* = 2.1 Hz, H-2) and 6.36 (1H, *d*, *J* = 2.1 Hz, H-4), and 1,2,4-trisubstituted benzene ring at δ 7.33 (1H, *d*, *J* = 9.0 Hz, H-5), 7.25 (1H, *dd*, *J* = 9.0 and 3.0 Hz, H-6), and 7.50 (1H, *d*, *J* = 3.0 Hz, H-8). From this informations concluded that compound **CC5** was a 1,3,7-trihydroxyxanthone.

Table 6 $^1\text{H-NMR}$ (300 MHz) spectral data of **CC5** in CDCl_3 and CD_3OD

Position	δ_{H} (mult., <i>J</i> in Hz)	Position	δ_{H} (mult., <i>J</i> in Hz)
1	–	6	7.25 (<i>dd</i> , <i>J</i> = 9.0 and 3.0)
2	6.25 (<i>d</i> , <i>J</i> = 2.1)	7	–
3	–	8	–
4	6.36 (<i>d</i> , <i>J</i> = 2.1)	8a	–
4a	–	9	–
5a	–	9a	–
5	7.33 (<i>d</i> , <i>J</i> = 9.0)	1-OH	12.92 (<i>s</i>)

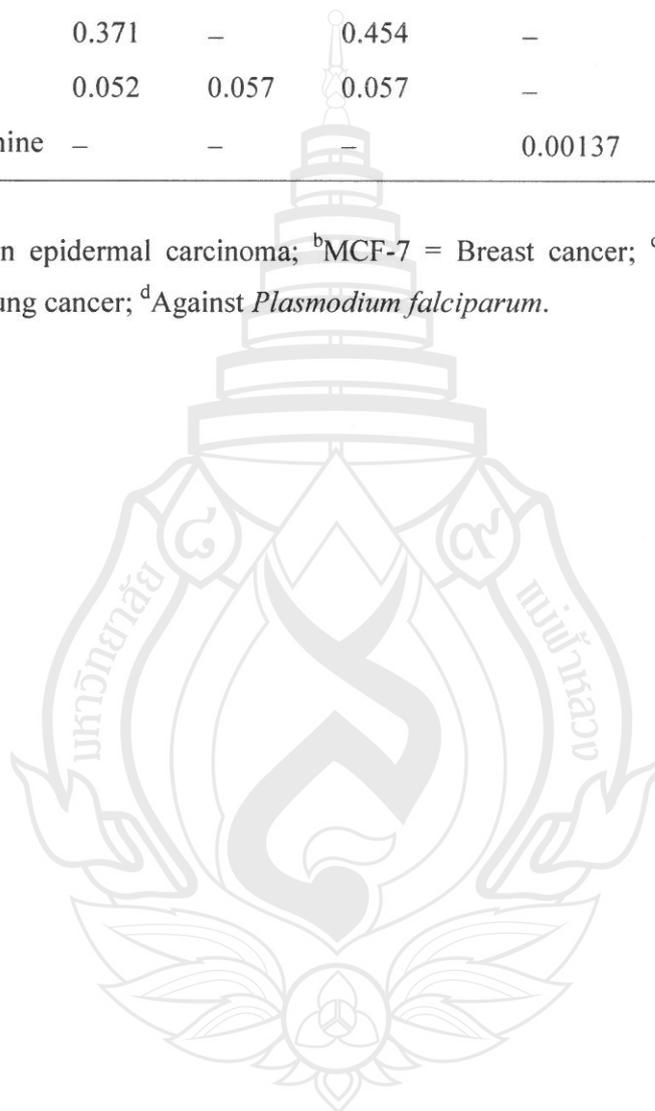
3.6 Biological evaluation of compounds **CC3** and **CC4**

As summarized in Table 7, all compounds **CC3** and **CC4** were evaluated for their antimalarial activity against *P. falciparum*, and cytotoxic activity against NCI-H187 (human lung cancer), KB (oral human epidermal carcinoma) MCF-7 (breast cancer) cancer cell lines. Compound **CC4** showed strong inhibitory effect against all three cancer cell lines while **CC3** was found to be moderate active (Table 7). Also, compounds **CC3** and **CC4** exhibited strong inhibitory effects against *P. falciparum*, with IC_{50} values of 2.10 and 0.66 $\mu\text{g/ml}$, respectively. It should be noted that the structural variation between compounds **CC3** and **CC4** corresponds to the remarkably different activity. The only difference between **CC3** and **CC4** is substituents at C-1 and C-2. Compound **CC4** contains a chromene ring at C-C1/C-2 but **CC3** has a hydroxyl and isoprenyl groups at C-1 and C-2, respectively. This chromene ring appears to be particularly responsible for the cytotoxic and antimalarial activity.

Table 7 Biological activity of Compounds **CC3** and **CC4**

Compound	Cytotoxic activity (IC ₅₀ , µg/ml)			Antimalarial activity ^d (IC ₅₀ , µg/ml)
	KB ^a	MCF-7 ^b	NCI-H187 ^c	
CC3	5.94	13.12	6.62	2.02
CC4	0.53	6.29	1.19	0.66
Elliticine	0.371	–	0.454	–
Doxorubicin	0.052	0.057	0.057	–
Dihydroartemisinin	–	–	–	0.00137

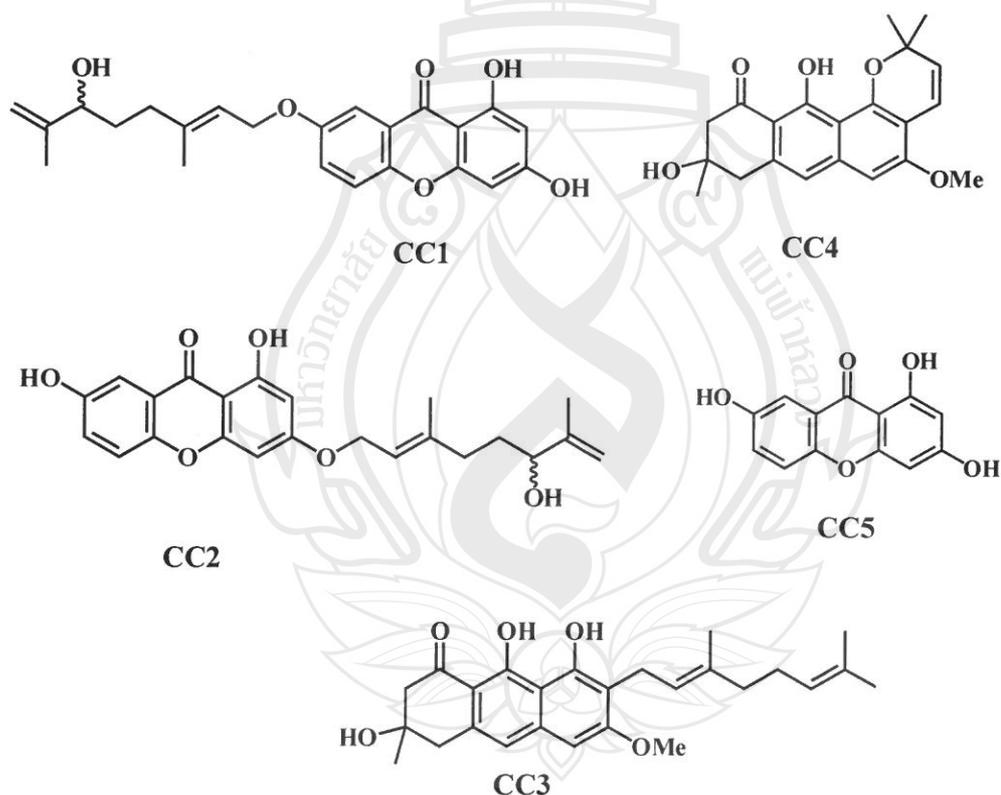
^aKB = Oral human epidermal carcinoma; ^bMCF-7 = Breast cancer; ^cNCI-H187 = human small cell lung cancer; ^dAgainst *Plasmodium falciparum*.



CHAPTER 4

CONCLUSION

The studies of chemical constituents from the fruits of *C. cochinchinense* led to isolation of five phenolic compounds (CC1-CC5). Two of them are new xanthons named cochinxanthon A (CC1) and B (CC2). Their structures were characterized using spectroscopic methods. Compounds CC3 and CC4 also studied on biological activity including anti cancer and antimalaria activity. Both of them were found to be active with anti-cancer activity against NCI-H187 (human lung cancer), KB (oral human epidermal carcinoma) MCF-7 (breast cancer) cancer cell lines and antimalarial activity against *P. falciparum*.



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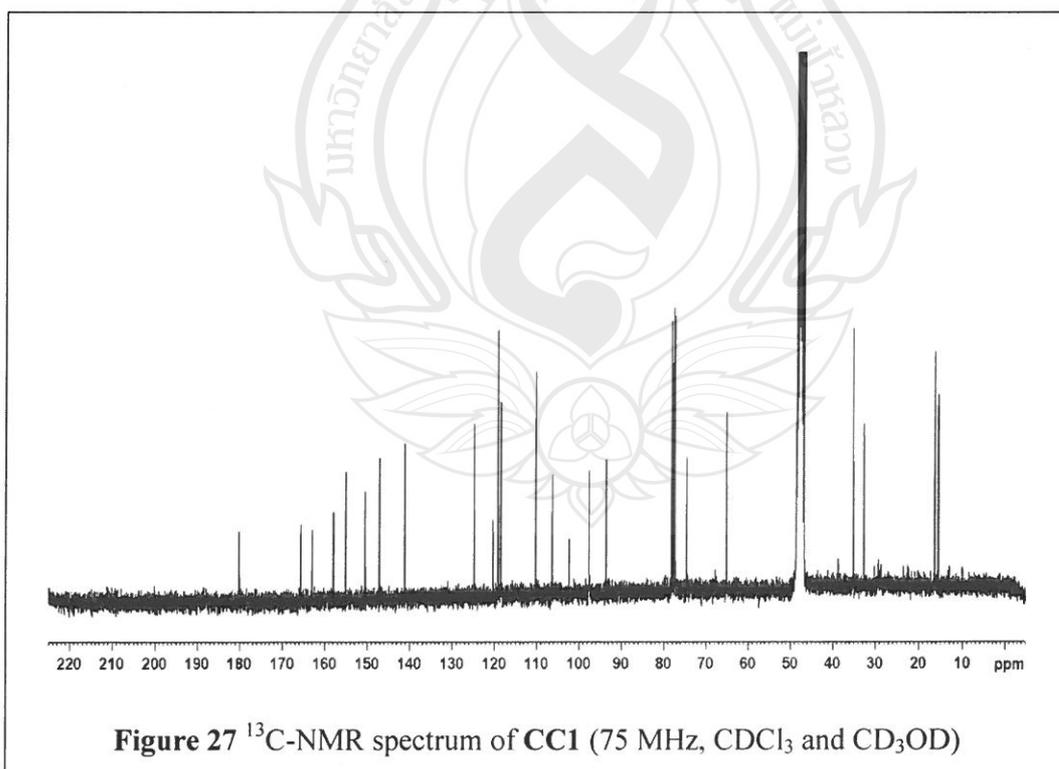
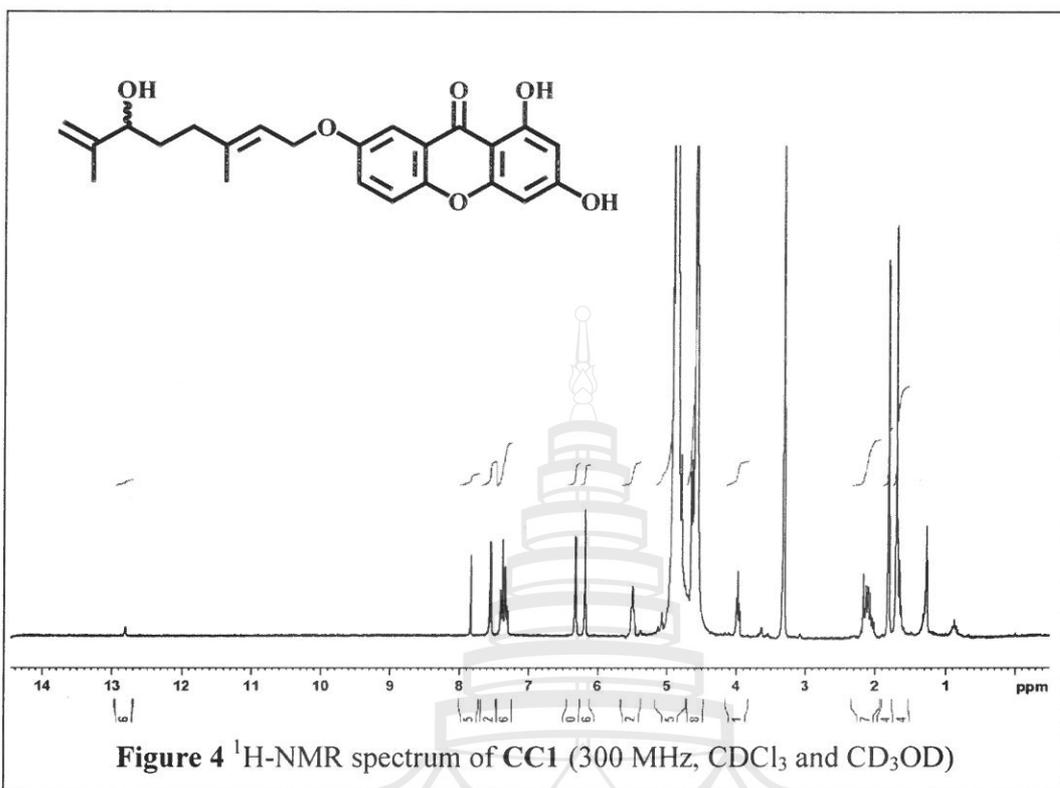
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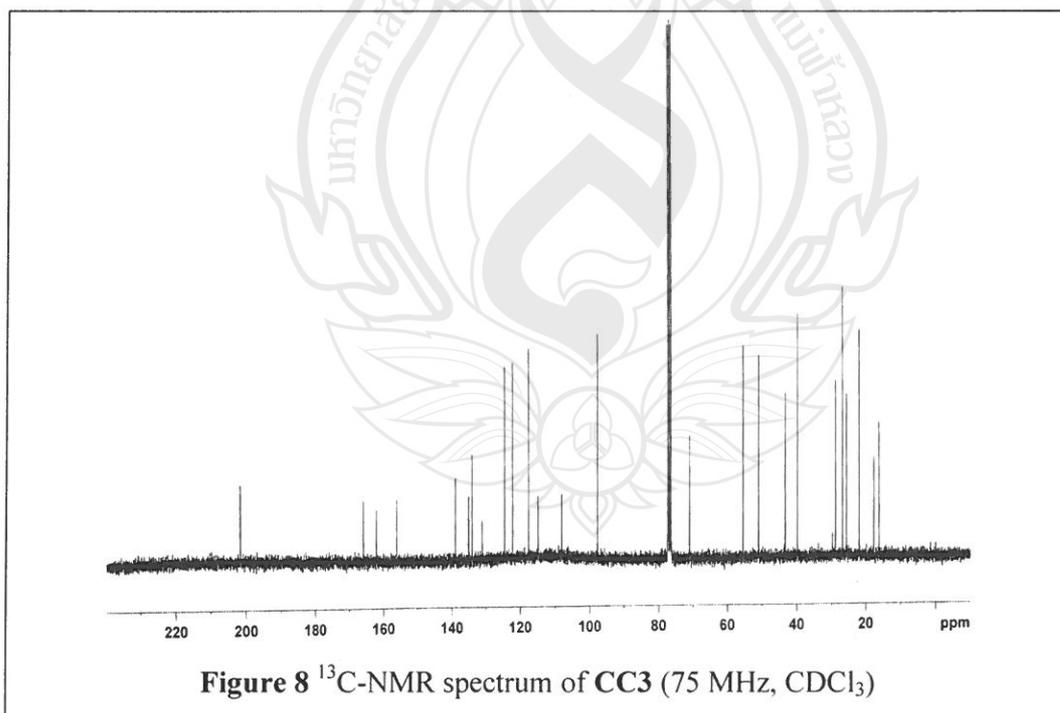
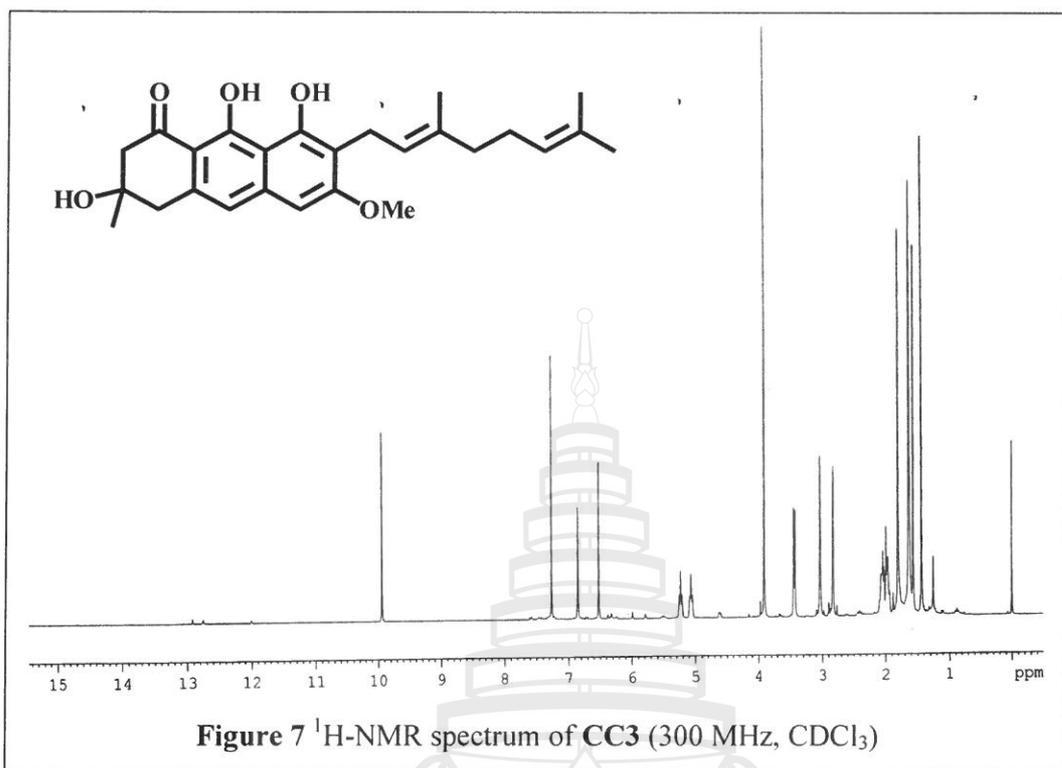
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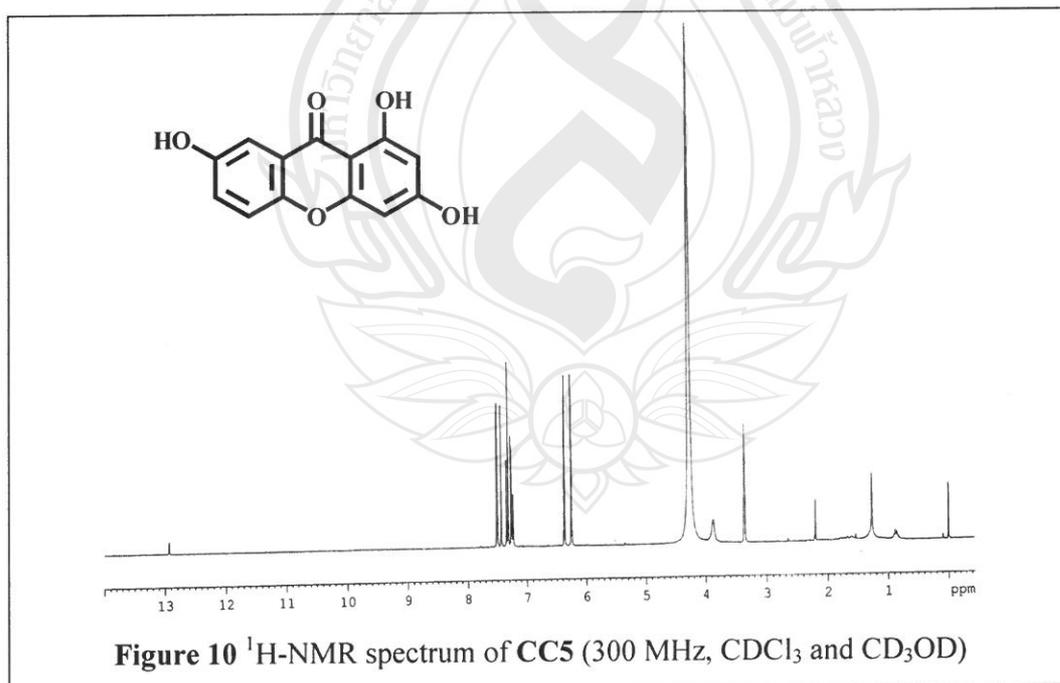
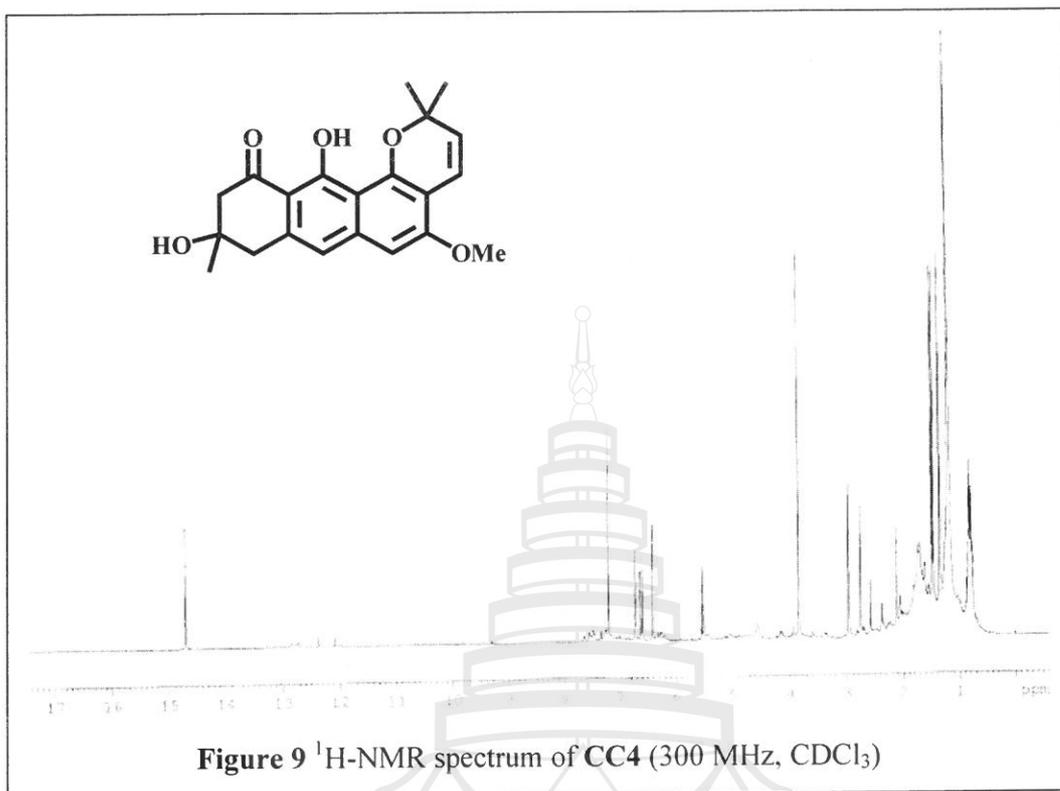


APPENDIX









BIOGRAPHY

1. Asst. Prof. Dr. Surat Laphookhieo

School of Science, Mae Fah Luang University, Muang, Chiang Rai

Education background

Level	Major	University	Year
Ph.D	Organic Chemistry	Prince of Songkla University	2005
M.Sc	Organic Chemistry	Prince of Songkla University	2002
B. Sc.	Chemistry	Rajabhat Institute Surat Thani	1999

2. Mr. Wisanu Maneerat

School of Science, Mae Fah Luang University, Muang, Chiang Rai

Education background

Level	Major	University	Year
B. Sc.	Biotechnology	Mae Fah Luang University	2008

3. Dr. Sorwaporn Koysomboon

Faculty of Art and Science, Prince of Songkla University, Muang, Surat Thani

Education background

Level	Major	University	Year
Ph.D	Chemistry	Walailak University	2007
B. Sc.	Chemistry	Prince of Songkla University	1999