Chemical Constituents from the Root of *Garcinia cowa* and Antioxidation Properties

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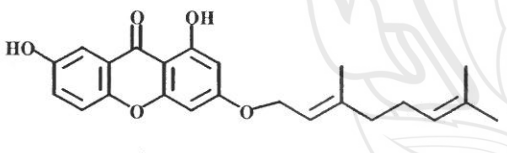
PREFACE

Garcinia cowa Roxb. (Guttiferae) which is one of the medicinal plants, was investigated for the chemical constituents and biological activities. It is a part of the basic research on the utilization of Thai medicinal plants. Many xanthones have been isolated from this plant. Some of xanthone compounds have been reported to have antimalarial and antimicrobial activity, no many study on the antioxidant potential of this group of natural products has been described. Natural antioxidants have attracted attention because some synthetic antioxidants have been found to be carcinogenic and harmful to lung and liver. Since most xanthones that occur in all higher plants and in various parts of the plant have phenolic functional groups on a linear tricyclic ring, they are anticipated to have antioxidant activity. So further study on the antioxidant potential of isolated compounds should be performed which can lead to active compounds. Therefore, Thai plant can be utilized as a natural resource of potential drugs.

ABSTRACT

Isolation of the chemical constituents from the root of *Garcinia cowa* Roxb., by chromatographic and crystallization techniques, yielded a new xanthone: 3-geranyloxy-1,7-dihydroxyxanthone (**1**) and twelve previously reported xanthones: cowaxanthone (**2**), cowanin (**3**), cowanol (**4**), mangostin (**5**), *β*-mangostin (**6**), 1,3,6-trihydroxy-7-methoxy-2,5-*bis*(3-methyl-2-butenyl)xanthone (**7**), 7-geranyloxy-1,3-dihydroxyxanthone (**8**), cochinchinone A (**9**), crytoxycochinchinone C (**10**), macluraxanthone (**11**), 10-*O*-methylmacluraxanthone (**12**), isocudraniaxanthone B (**13**), stigmasterol (**14**). Their structures were elucidated on the basis of UV, IR and NMR spectroscopic data.

The acetone and crude methanolic extracts exhibited the antioxidative activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay with IC_{50} 11.25 and 17.85 μg/mL, respectively. Compound **11** and **13** showed strong activity (IC_{50} 7.40 and 6.50 μM, respectively) than that of BHT (IC_{50} 8.70 μM) whereas the other (**1-10** and **12**) compounds showed weak activity.



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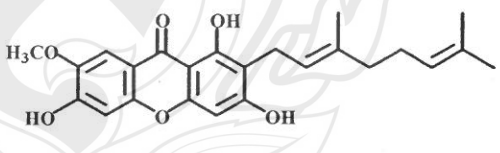

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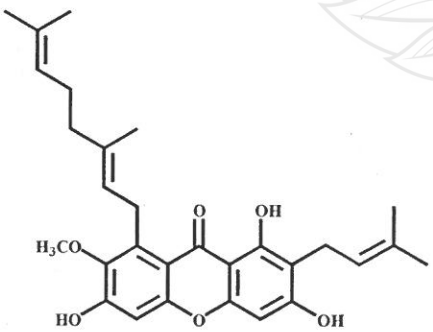

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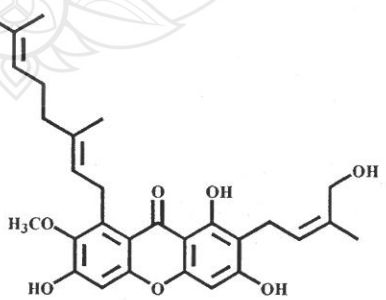

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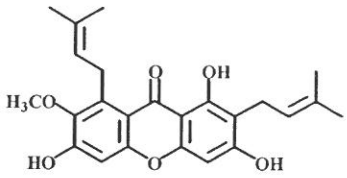

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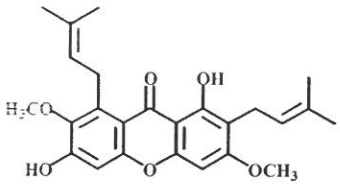

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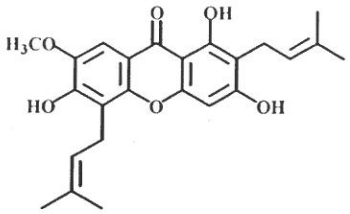

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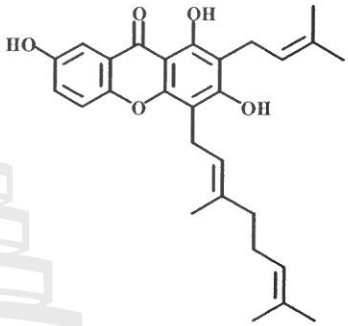

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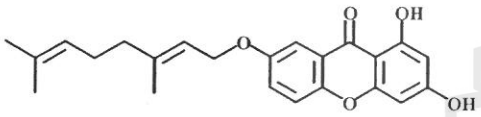

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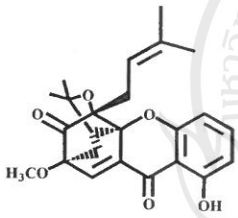

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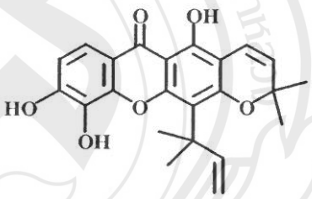

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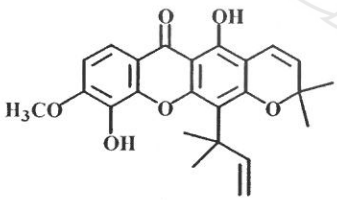

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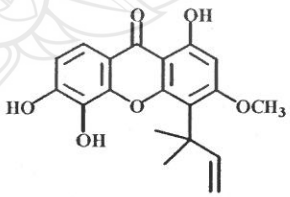

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Suwanna Deachathai

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ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
m	=	*multiplet*
dd	=	*doublet of doublet*
dt	=	*doublet of triplet*
br s	=	*broad singlet*
br m	=	*broad multiplet*
g	=	gram
kg	=	kilogram
mg	=	miligram
µg	=	microgram
mM	=	millimolar
mL	=	milliliter
h	=	hour
min	=	minute
%	=	percent
nm	=	nanometer
cm^3	=	cubic centimeter
m.p.	=	melting point
cm^{-1}	=	reciprocal centimeter (wave number)
δ	=	chemical shift relative to TMS
J	=	*coupling constant*
$[\alpha]_D$	=	specific rotation

λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ε	=	molar extinction coefficient
m/z	=	a value of mass divided by charge
^{o}C	=	degree celcius
MHz	=	Megahertz
ppm	=	part per million
c	=	concentration
MS	=	Mass Spectroscopy
EIMS	=	Electron Impact Mass Spectrometry
FABMS	=	Fast Atom Bombardment Mass Spectrometry
UV	=	Ultraviolet-Visible
IR	=	Infrared
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimentional Nuclear Magnetic Resonance
COSY	=	Correlated Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
NOE	=	Nuclear Overhauser Effect Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
CH_2Cl_2	=	dichloromethane
$CHCl_3$	=	chloroform
EtOAc	=	ethyl acetate
Me_2CO	=	acetone

MeOH	=	methanol
TMS	=	tetramethylsilane
Acetone-d_6	=	hexadeuteroacetone
DMSO-d_6	=	hexadeuterodimethyl sulphoxide
$CDCl_3$	=	deuterochloroform
CD_3OD	=	tetradeuteromethanol
D_2O	=	deuterium oxide
IC_{50}	=	50% Inhibition Concentration
DPPH	=	2,2-diphenyl-1-picrylhydrazyl radical
BHT	=	butylated hydroxy toluene

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 activities. The important process is the application of the knowledge in pharmacology and medicine.

1.2 Objectives

Analytical TLC of the root extract of *G. cowa* indicated that some more chemical constituents in the root extract are of interest. The preliminary testing on radical scavenging of the crude material also exhibits potent activity. It then prompted us to investigate the components of *G. cowa* more thoroughly and search for the antioxidation activity of pure compounds.

1.3 Scope of study

Extraction and isolation of secondary metabolite from the root of *G. cowa*, Characterization of all isolates by spectroscopic methods (UV, IR and NMR) and evaluation of antioxidation activity of crude extract and pure compounds.

1.4 Benefit

Garcinia cowa which is one of the medicinal plants, was investigated for the chemical constituents and biological activities. It is a part of the basic research on the utilization of Thai medicinal plants. Many xanthones have been isolated from this plants. Many compounds of this types have been reported to have antimicrobial, cytotoxic, antitumor and antimalarial activities. So further study on the biological activity of the isolated compounds should be performed which can lead to active compounds. Therefore, some active compounds might be applied into the cosmetic, pharmacy or agriculture. This work might be published in international journals.

CHAPTER 2

LITERATURE REVIEWS

Garcinia cowa Roxb. known as Cha-muang, belongs to the Guttiferae family. It grows widely in the tropical rain forest area. *G. cowa* is an erect, small to medium tree, reaching 60 feet tall. The trunk is simple straight, the branches are slender and lower reaching the ground. It has dark-gray bark, inner bark with opaque, lemon yellow exudate. The leaves are broad-lanceolate acute at both ends dark green beneath 3-5 by 1-2 inches size, the veins are 0.1-0.15 inches apart, its slender, regular and inarching with an intra-marginal one, drying pinkish grey-brown, the young leaves are red and edible. Male flowers are in 3-8 flowered which rarely auxillary umbels, male pedicels are 0.16-0.33 inches, sepals are 0.16 inches in length, broad-ovate, with a thick fleshy, the colour of sepal is yellow and pink on both surfaces. Petals twice as long as with sepals and oblong. Stamens numerous forming a quadrate mass, with a rudimentary stigma O, anthers in a shortly subsessile, 4-celled. Hermaphroditic flower is solitary which rarely 2-3 at axillary and sessile. Ovary is subglobose, with stigmatic rays spreading and papillose. Stamens (sterile) in 4 clusters of 3-8 unequal filaments. Fruit have orange or dark-yellow of small size, drying jet black, with 4-8 grooved to the top with a tip mamilla (Hooker, 1875). Seeds embedded in pale orange pulp.

Figure 1 *Garcinia cowa* Roxb.

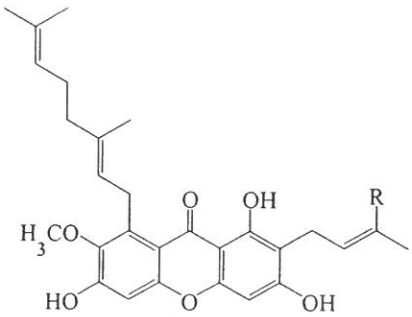
The *Garcinia cowa* was first investigated by Krahn in 1968. Cowanin, cowanol and cowaxaanthone were isolated from the stem. Further study by Lee and Chan in 1977, 1,3,6-trihydroxy-7-methyoxy-8-(3,7-dimethyl-2,6-octadienyl)xanthone was isolated. In 1994, Na Pattalung isolated 1,3,6-trihydroxy-7-methyoxy-2,5-bis(3-methyl-2-butenyl)xanthone and norcowanin from the latex and in 1977, 7-*o*-methylgarcinone E and *β*-mangostin were reported to obtained from the stem bark by Likhitwitayawuid. In 2005, Mahabusarakam isolated cowagarcinone A-E from the latex. In addition, cowaxanthone A-E were reported to obtain from the fruits by Panthong (2006).

Garcinia cowa has been used in the folk medicine for variety purposes. The bark has been used in treaditional medicine as an antipyretic, antimicrobial agent (Na Pattalung, *et al.*, 1994), in addition it was used as pesticide and mosquito larvicide (Maikhuri and Gangwar 1993). The sun dried fruit was used to treat dysentery (Rao, *et al.*, 1981). The methanolic extract of leaves (fresh and dried) has been reported to show strong antitumor-promoting activity (Maurakami, *et al.*, 1995 and 1997). The latex from trungs and the roots are used as antifever agent (Na Pattalung, *et al.*, 1994). The pure compounds (cowanin, cowanol, cowaxanthone, 7-*o*-methylgarcinone E and *β*-mangostin) and 95% aqueous ethanols of dried stem bark have been reported to have antimalarial activity (Likhitwitayawuid, *et al.*, 1998) Xanthones isolated from *G. cowa* were summarized in **Table1**.

Table 1 Xanthones from *Garcinia cowa* Roxb.

Part	Xanthone compound	Structure	Reference
Latex	cowanin	1	Na Pattalung, *et al.*,
	cowanol	2	1994
	cowaxanthone	3	
	1,3,6-tri(OH)-7-(OMe)-2,5-bis (3-(Me)-2-butenyl)xanthone	4	
	cowagarcinone A-E	9-13	Mahabusarakam, *et al.*, 2005
	norcowanin	7	
Stem	cowanin	1	Krahn, 1968
	cowanol	2	
	cowaxanthone	3	
	1,3,6-tri(OH)-7-(OMe)-8-(3,7-di (Me)-2,6-octadienyl)xanthone	5	Lee and Chan, 1977
	7-*O*-methylgarcinone E	6	Likhitwitayawuid, *et al.*, 1997
Stem bark	*β*-mangostin	8	Likhitwitayawuid,
	cowanin	1	*et al.*, 1998
	cowanol	2	
	cowaxanthone	3	
Fruit	cowaxanthone A-E	14-18	Panthong, *et al.*, 2006

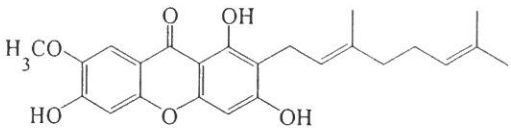
The structure of xanthones from *G. cowa* Roxb.



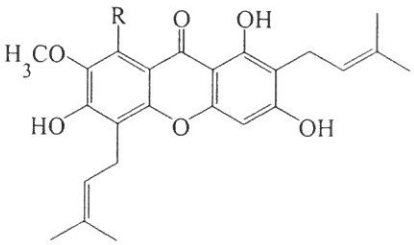
1 R = CH_3 : cowanin

2 R = CH_2OH : cowanol

13 R = CH_2OAc : cowagarcinone E



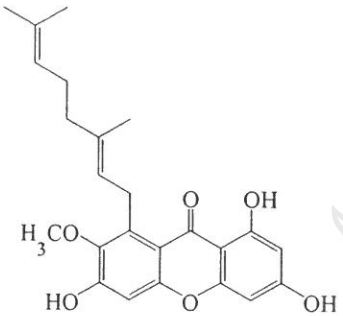
3 cowaxanthone



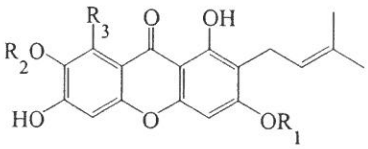
4 R = H : 1,3,6-tri(OH)-7-(OMe)-2,5-bis (3-(Me)-2-butenyl)xanthone

6 R = prenyl : 7-*O*-methylgarcinone E

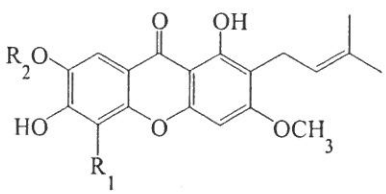
9 R = geranyl : cowagarcinone A



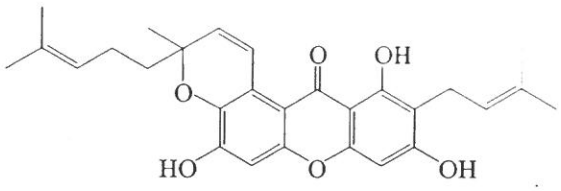
5 : 1,3,6-tri(OH)-7-(OMe)-8-(3,7-di(Me)-2,6-octadienyl))xanthone



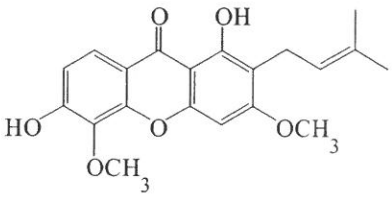
	R_1	R_2	R_3	
7	H	H	geranyl	: norcowanin
8	CH_3	CH_3	isoprenyl	: β-mangostin



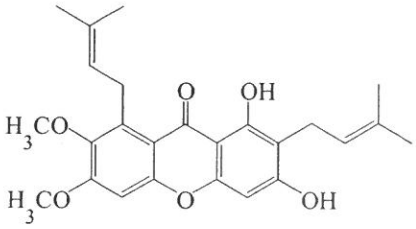
	R_1	R_2	
10	H	CH_3	: cowagarcinone B
11	CH_3	H	: cowagarcinone C



12 cowagarcinone D



14 cowaxanthone **A**



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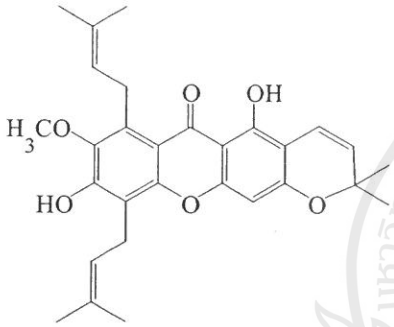
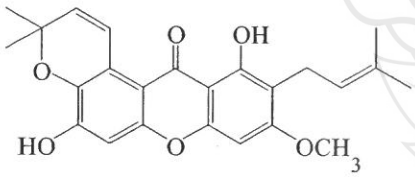
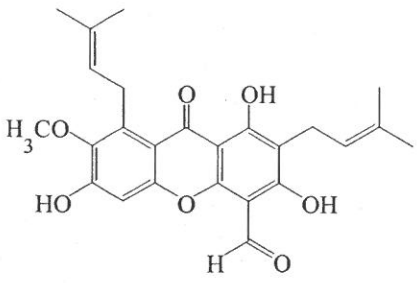


16 cowaxanthone **C**



17 cowaxanthone **D**



18 cowaxanthone **E**

Although a number of biological properties of xanthones from *G. cowa* Roxb., such as antimalarial, antimicrobial activities have been recognized, no study on the antioxidant potential of the chemical constituents from the root of *G. cowa*. Natural antioxidants have attention because some synthetic antioxidants have been found to be carcinogenic and harmful to lungs and liver (Yamasaki, *et al.*, 1994). Since most of xanthones that occur in all higher plants and various parts of the plants have phenolic functional groups on a linear tricyclic ring, they are anticipated to have antioxidant activities. In addition, several xanthones from *Garcinia* genus have been reported an antioxidation activity such as 1,4,5-trihydroxy-3,6-bis(3-methyl-2-butenyl)xanthone, 1,2,5-trihydroxyxanthone, garciniaxanthones F, G and H from the wood of *Garcinia subelliptica* (Minami, *et al.*, 1994 and 1996).

CHAPTER 3

METHODOLOGY

3.1 General method

Melting points were recorded in °C and were measured on a digital Fisher-John melting point apparatus. Infrared spectra were recorded by using Perkin-Elmer FTS FT-IR spectrometer. Major bands (λ_{max}) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. The ^{1}H and ^{13}C NMR spectra were recoreded on 300 and/or 400 MHz Bruker FTNMR Ultra Shield™ spectrometer. Spectra were recorded in deuterochloroform tetradeutero-methanol or hexadeutero-dimethyl sulphoxide solution and were recorded as δ value in ppm down field from TMS (internal standard δ 0.00). Optical rotation was measured in methanol solution with sodium D line (590 nm) on a JASCO P-1020 polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use. Solvents for crystallization were analytical grade reagent. Pre-coated TLC aluminum sheets of silica gel 60 PF_{254} (20x20 cm, layer thickness 0.2 mm) were use for analytical proposes and the compounds were visualized under ultraviolet light and/or vanillin-sulfuric acid reagent. Plates of silica gel PF_{245}, 20x50 cm, thickness 1.25 nm, activated at 110 °C for 3 h were utilized in the case of preparative TLC. Quick column chromatography was performed on silica gel 60H (Merck). Column chromatography was performed by using silica gel (Merck) type 100 (70-230 mesh ASTM). DPPH (Fulka) were used for antioxidation activity testing and the absorbance were measured by SP 1104 spectrophotometer.

3.2 Plant material

The root of *Garcinia cowa* Roxb. (Guttiferae) was collected from Lipung district, amphur Palein, Trang province in the Southern part of Thailand. A specimen has been deposited in the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand.

3.3 Extraction and Isolation

Root of *G. cowa* 7.0 kg was extracted by immersed at room temperature with acetone (12 days) and methanol (20 days), respectively. After evaporation, the viscous crude acetone (111.45 g) and methanolic (611.26 g) extracts were obtained. The process of extraction was shown in **Figure 2**.

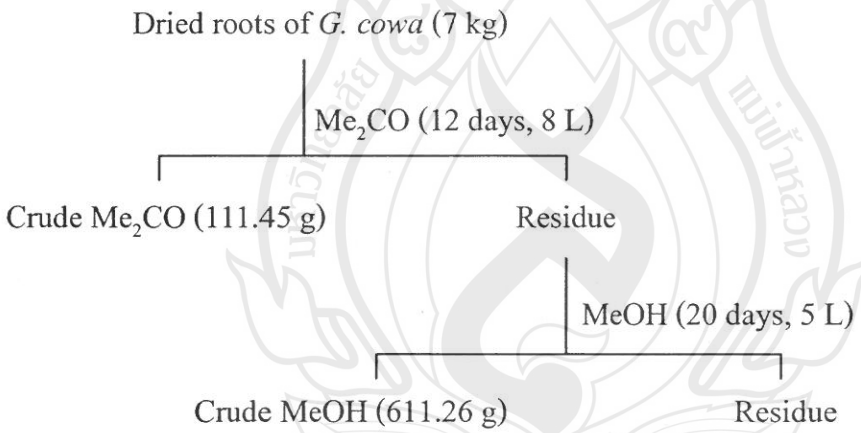


Figure 2 Extraction of crude acetone and crude methanolic extracts from the root of *G. cowa*

Crude acetone (100.50 g) was subjected to QCC using silica gel as the stationary phase and eluted with hexane, hexane-CH_2Cl_2, CH_2Cl_2, CH_2Cl_2-Me_2CO and Me_2CO. On the basis of their TLC characteristics, the collected fractions (200 mL each) which contained the same major components were combined to afford fractions B1-B30 (**Figure 3**).

Fraction B7 (0.84 g) was purified by CC and eluted with 50% CH_2Cl_2-hexane. Fractions with the similar TLC chromatograms were combined to afford seven fractions (B7.1-B7.7). Fraction B7.4 which contained one major component was recrystallized in the mixture of hexane-CH_2Cl_2 (2:3) to give **5** (7.0 mg). Fraction B7.5 contained two major components was recrystallized in the mixture of hexane-CH_2Cl_2 (2:3) to give yellow solid **6** (44.8 mg) and **7** (16.6 mg). Fraction B7.7 which contained one major components was recrystallized in the mixture of hexane-CH_2Cl_2 (2:3) to give yellow solid **8** (40.6 g).

Fraction B9 (1.43 g) was fractionated by CC and eluted with 50% of CH_2Cl_2 in hexane to afford 7 fractions (B9.1-B9.7). Fraction B9.2 contained two major components was re-column chromatography and eluted with 50% CH_2Cl_2-hexane. Fractions with the similar TLC chromatograms were combined to afford 8 fractions (B9.2.1-B9.2.8). Fraction B9.2.2 and fraction B9.2.5 were further purified by CC to give **9** (3.6 mg) and **10** (27.9 mg), respectively. Fractions B9.5 and B9.6 were re-crystallized in the mixture of hexane-CH_2Cl_2 (2:3) to give yellow solid **11** (20.2 mg) and **12** (64.9 mg). The filtrate from the fractions B9.5 and B9.6 were combined and re-crystallization to give **12** (218.3 mg).

Fractions B12 and B13 were crystallized in the mixture of hexane-CH_2Cl_2 (1:4) to give yellow solid **3** (0.21 g) and **2** (0.29 g).

Fraction B16 (6.8133 g) was purified by CC and eluted with 90% CH_2Cl_2-hexane. Fractions with the similar TLC chromatograms were combined to afford 14

fractions (B16.1-B16.14). Fraction B16.4 contained one major component **3** (59.3 mg). Fraction B16.5 contained two major components was re-column chromatography and elute with 90% CH_2Cl_2-hexane to afford **1** (13.5 mg) and **4** (6.8 mg).

Fraction B17 (5.4717 g) was subjected to CC and eluted with CH_2Cl_2 to give **14** (2.45 g).

Fraction B18 (264.67 mg) was fractionated by CC and eluted with 30% CH_2Cl_2-hexane to give **1** (58.6 mg).

Fraction B19 (1.0473g) was future purified by CC and eluted with 40% CH_2Cl_2-hexane to give **11** (0.65 mg), **12** (350.3 mg) and **13** (0.55 mg) as yellow solid.

Fraction B22 (1.22 g) was further subjected to CC and eluted with 45% CH_2Cl_2-hexane. Fraction with the similar TLC chromatograms was combined to afford 13 fractions (B22.1-B22.13). Fraction B22.6 contained one major component was crystallized in the mixture of hexane-CH_2Cl_2 (4:1) to give **10** (0.32 mg) and **13** (0.25 mg).

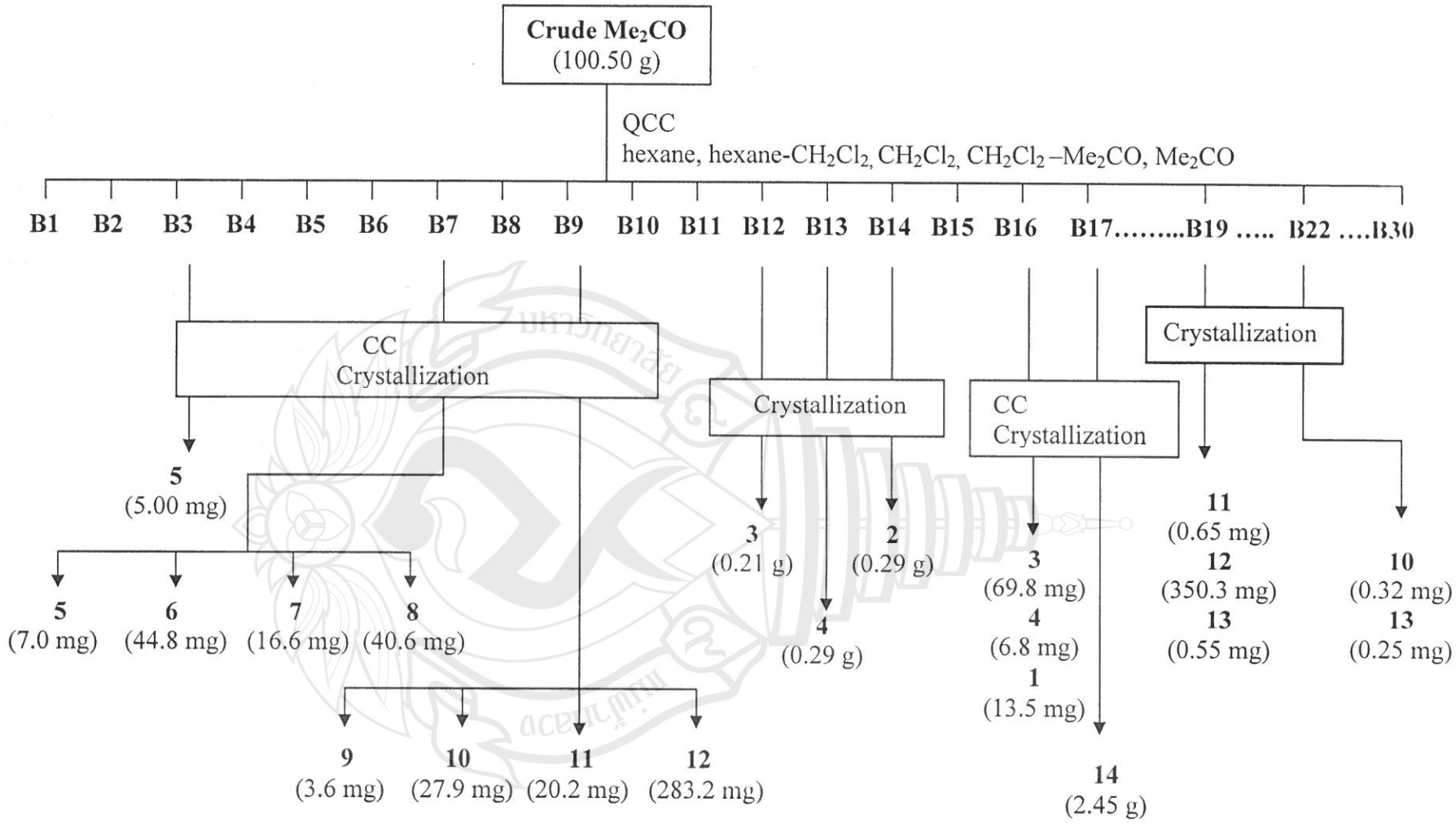


Figure 3 Isolation of compounds **1-14** from the root of *G. cowa*

3.4 Estimation of the antioxidation activity

The potential antioxidation properties of the crude material and pure compounds isolated from the root of *G. cowa* were assessed on the basis of the scavenging activity of the stable α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical.

3.4.1 Screening on the free radical scavenging activity of the crude extracts

The crude material was dissolved in absolute ethanol to prepare the solution with the concentration of 100 μg/mL. The solution of each sample (50 μL) was mixed with 0.05 mM DPPH ethanolic solution (3 mL) in a cuvette at room temperature. The trapping effect by measuring the absorbance changes of the solution at 517 nm against 0.05 mM DPPH ethanolic solution every 15 min. The measurements were performed at least in triplicate. The degree of loss of color implied the activity. The residual absorbance at 517 nm were shown in **Table 2**.

Table 2 The average absorption of the crude extracts.

(at final concentration 100 μg/mL)

Sample	average absorbance at 517 nm.			
	0 min	15 min	30 min	45 min
DPPH	0.640	0.640	0.640	0.620
Acetone extract	0.352	0.532	0.078	0.071
Methanolic extract	0.532	0.532	0.074	0.078

3.4.2 Evaluation of IC_{50} value of the crude material

The ethanolic solutions of crude material with the concentration of 20, 40, 60, 80 and 100 µg/mL were prepared. The solution (50 µL) was mixed with the ethanolic solution of DPPH (0.05 mM, 3 mL). The mixed solution was allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm. BHT and ascorbic acid were used for a positive control. The measurements were performed at least in triplicate. The results were shown in **Table 3**. The absorbance at each time point was plotted against the concentration. The concentration that required to scavenge 50% DPPH free radical was the IC_{50}.

IC_{50} (the concentration of the sample at 50% inhibition) was obtained by linear regression analysis of dose response curve, which was plotted between % inhibition and concentration (µg/mL).

$$\% \text{ Inhibition} = \frac{A\text{control} - A\text{sample}}{A\text{control}} \times 100$$

Table 3 The average absorption and % inhibition of the crude extracts at various concentrations.

concentration (µg/mL)	Acetone extract		Methanolic extract	
	absorbance	% inhibition	absorbance	% inhibition
control (DPPH)	0.531	0.00	0.531	0.00
20	0.145	72.69	0.227	57.25
40	0.069	87.01	0.056	87.45
60	0.033	93.79	0.052	90.21
80	0.021	96.05	0.048	90.96

3.4.3 Free radical scavenging activity of pure compounds

The testing was performed as in 3.4.2 except the final concentration of the solution was made at 10 μM. The results were shown in **Table 4**.

Table 4 The average absorption and % inhibition of pure compounds

Sample	10 μM	
	absorbances	% inhibition
control (DPPH)	0.573	-
1	0.566	1.22
2	0.563	1.75
3	0.570	0.52
4	0.565	1.40
5	0.554	3.32
6	0.562	1.92
7	0.571	0.35
8	0.571	0.35
9	0.568	0.87
10	0.563	1.75
11	0.180	68.59
12	0.554	3.32
13	0.165	71.20
BHT	0.285	50.26
Ascorbic acid	0.107	81.33

CHAPTER 4

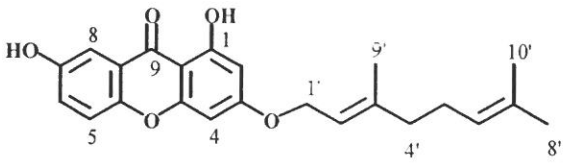
RESULTS AND DISCUSSION

4.1 Structural determination

The root of *Garcinia cowa* was extracted with acetone and methanol, respectively. After evaporation, the viscous crude acetone extract (111.45 g.) and crude methanolic extract (611.26 g) were obtained.

Crude acetone (100.50 g) was separated by QCC over silica gel, eluting with hexane, hexane-CH_2Cl_2, CH_2Cl_2, CH_2Cl_2-Me_2CO and Me_2CO in a polarity gradient manner to give 30 fractions. Selected fractions were further purified by column chromatography, preparative TLC and/or crystallization to afford yielded a new xanthone: 3-geranyloxy-1,7-dihydroxyxanthone (**1**) and twelve previously reported xanthones: cowaxanthone (**2**), cowanin (**3**), cowanol (**4**), mangostin (**5**), β-mangostin (**6**), 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone (**7**), 7-geranyloxy-1,3-dihydroxyxanthone (**8**), cochinchinone A (**9**), cratoxycochinchinone C (**10**), macluraxanthone (**11**), 10-*O*-methylmacluraxanthone (**12**), isocudraniaxanthone B (**13**), stigmasterol (**14**). The structures were elucidated by spectroscopic methods.

1: 3-Geranyloxy-1,7-dihydroxyxanthone



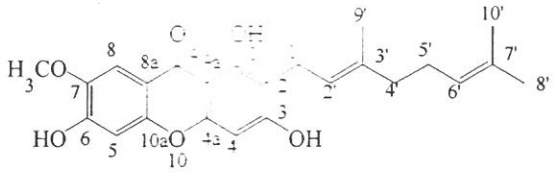
Compound **1** was a yellow solid, m.p. 147-148 °C. The UV spectrum showed maximum absorption bands at 203, 229, 259, 307 and 374 nm. The IR spectrum showed the absorption band of a conjugated carbonyl group at 1647 cm^{-1} and a hydroxyl group at 3288 cm^{-1}. The ^{1}H NMR spectrum (**Table 5**) exhibited signals of a hydrogen-bonded hydroxyl proton at δ 12.70 (*s*, 1-OH) and three aromatic protons which coupled as an ABX system at δ 7.29 (*d*, *J* = 9.0 Hz, H-5), 7.24 (*dd*, *J* = 9.0, 3.0 Hz, H-6), and 7.58 (*d*, *J* = 3.0 Hz, H-8). The resonances at δ 6.39 (*d*) and 6.33 (*d*) with a coupling constant of 3.0 Hz were of *meta* aromatic protons H-4 and H-2, respectively. The presence of a geranyl unit was observed from the characteristic signals of protons at δ 4.62 (*d*, H-1′), 5.48 (*t*, H-2′), 2.11 (*m*, H-4′), 2.12 (*m*, H-5′), 5.09 (*t*, H-6′), 1.55 (*s*, H-8′), 1.76 (*s*, H-9′) and 1.67 (*s*, H-10′). The evidence from the chemical shift of H-1′ (δ 4.62) indicated that the geranyl group formed bond to electron withdrawing atom which was assigned for an oxygen atom. Furthermore, the location of the *o*-geranyl side chain was assigned at C-3 from the HMBC correlations of H-1′ to C-3 and C-2′; H-4 to C-3 and the differential NOE technique by irradiation of the signals of H-2 and H-4 which affected the signal of H-1′. The presence of five oxygenated aromatic carbons was deduced from the ^{13}C NMR data at δ 166.2 (C-3), 163.2 (C-1), 157.5 (C-4a), 152.3 (C-7) and 150.7 (C-4b). Thus, 3-geranyloxy-1,7-dihydroxyxanthone was assigned for compound **1**, which was isomer of compound **8**. It is a new xanthone derivative.

Table 5 The NMR spectral data of **1**

Position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	HMBC
1	163.2 (C)	-	-
2	97.6 (CH)	6.33 (1H, *d*, 3.0 Hz)	C-1, C-4, C-9a
3	166.2 (C)	-	-
4	93.2 (CH)	6.39 (1H, *d*, 3.0 Hz)	C-2, C-3, C-4a, C-9a
4a	157.5 (C)	-	-
5	119.1 (CH)	7.29 (1H, *d*, 9.0 Hz)	C-7, C-8, C-8a
6	124.2 (CH)	7.24 (1H, *dd*, 9.0, 3.0 Hz)	C-5, C-7, C-8
7	152.3 (C)	-	-
8	109.1 (CH)	7.58 (1H, *d*, 3.0 Hz)	C-5, C-6, C-7, C-9
8a	120.7 (C)	-	-
9	180.7 (C=O)	-	-
9a	103.6 (C)	-	-
10a	150.8 (C)	-	-
1′	65.6 (CH_2)	4.62 (2H, *d*, 6.6)	C-3, C-2′, C-3′
2′	118.3 (CH)	5.48 (1H, *t*, 6.6)	C-4′, C-9′
3′	142.1 (C)	-	-
4′	39.5 (CH_2)	2.11 (2H, *m*)	C-2′, C-3′, C-5′
5′	26.3 (CH_2)	2.12 (2H, *m*)	C-3′, C-4′, C-6′
6′	123.6 (CH)	5.09 (1H, *t*, 6.6)	C-5′, C-10′
7′	132.0 (C)	-	-
8′	17.7 (CH_3)	1.55 (3H, *s*)	C-6′, C-7′, C-10′
9′	16.8 (CH_3)	1.76 (3H, *s*)	C-2′, C-3′, C-4′
10′	25.7 (CH_3)	1.67 (3H, *s*)	C-6′, C-7′, C-8′
1-OH	-	12.70 (1H, *s*)	-

* Carbon type was deduced from DEPT experiments.

2: Cowaxanthone



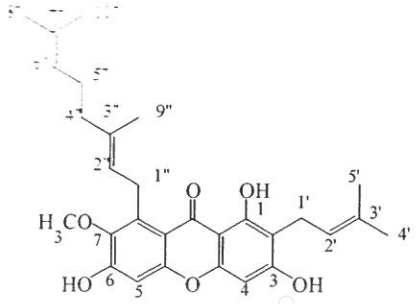
Compound **2** is a yellow crystal, m.p. 196-197 °C. The UV spectrum showed maximum absorption bands at 362, 321, 258, 242 and 210 nm. The IR spectrum showed the broad absorption band of O-H stretching at 3520 cm^{-1} and the sharp band of C=O stretching at 1635 cm^{-1}. The ^1H NMR spectrum (**Table 6**) exhibited a sharp *singlet* signal of a chelated hydroxyl proton C1-OH at δ 13.00 and three *singlet* signals of three isolated aromatic protons at δ 7.30, 6.66 and 6.21. The most deshielded aromatic proton signal, δ 7.30, was assigned for a signal of H-8 according to an isotropic effect by the carbonyl group. The other two aromatic proton signals, δ 6.66 and 6.21 were assigned for H-5 and H-4, respectively. The sharp *singlet* resonance at δ 3.74 belonged to the methoxy group and it was located at C-7. The remaining proton signals were assigned for geranyl side chain which was located at C-2. Those signals were assigned as follow; three *singlet* signals at δ 1.56, 1.40 and 1.33 were of three vinylic methyl groups, a *doublet* signal at δ 3.12 was assigned for benzylic methylene protons H-1', two sets of *multiplet* signals at δ 1.79-1.84 and 1.70-1.74 were the signals of two groups of methylene protons H-5' and H-4' and two sets of broad *triplet* signals at δ 5.04 and δ 4.84 were the signals of two olefinic methine protons H-2' and H-6'. Therefore, the structure of **2** was assigned to be 1,3,6-trihydroxy-7-methoxy-2-(3,7-dimethyl-2,6-octadienyl)xanthone. The proposed structure, the spectral data and the melting point were found to be corresponded to the previously isolated compound, cowaxanthone (Likhitwitayawuid, *et al.*, 1997).

Table 6 The ^{1}H NMR spectral data of **2**

Position	**2** δ_H (multiplicity, J_{HZ})	[A]**cowaxanthone** δ_H (multiplicity, J_{HZ})
1	13.00 (*s*, 1H)	14.34 (*s*, 1H)
3	-	10.82(*br s*, 1H)
4	6.21 (*s*, 1H)	6.30 (*s*, 1H)
5	6.66 (*s*, 1H)	6.87 (*s*, 1H)
6	-	10.82 (*br s*, OH)
7-OCH_3	3.74 (*s*, 3H)	3.86 (*s*, 3H)
8	7.30 (*s*, 1H)	7.41 (*s*, 1H)
1'	3.12 (*br d*, 7.0 Hz, 2H)	3.21 (*br d*, 2H, 7.0 Hz)
2'	5.04 (*br t*, 7.0 Hz, 1H)	5.17 (*br t*, 1H , 7.0 Hz)
4'	1.70-1.74 (*m*, 2H)	1.89 (*m*, 2H)
5'	1.79-1.84 (*m*, 2H)	1.98 (*m*, 2H)
6'	4.84 (*br t*, 7.0 Hz, 1H)	5.04 (*br t*, 7.0 Hz, 1H)
8'	1.33 (*s*, 3H)	1.49 (*s*, 3H)
9'	1.56 (*s*, 3H)	1.71 (*s*, 3H)
10'	1.40 (*s*, 3H)	1.55 (*s*, 3H)

[A]300 MHz, in DMSO-d_6

3: Cowanin



Compound **3** is a yellow solid, m.p. 136–137 °C. The UV spectrum showed maximum absorption bands at 355, 316, 255, 242 and 212 nm. The IR spectrum showed the absorption bands of conjugated carbonyl group at 1636 cm^{-1} and hydroxyl group at 3425 cm^{-1}. The ^1H NMR spectrum (**Table 7**) exhibited a resonance of a chelated hydroxyl group 1-OH at δ 13.70. Two *singlets* in aromatic region, δ 6.83 and 6.30 were assigned to be the signals of isolated proton H-5 and H-4, respectively. The presence of a methoxy group was shown at δ 3.80 as a *singlet* resonance and its was located at C-7. The ^1H NMR spectrum further revealed a characteristic signal of prenyl unit, of which the signal of *gem*-dimethyl protons resonated as two *singlets* at δ 3.45 and 1.77, a *doublet* due to benzylic methylene protons H-1' were at δ 3.45 and a *broad triplet* of an olefinic methine proton H-2' was at δ 5.29. The remaining signals were assigned for geranyl group. A *doublet* signal at δ 4.10 and a *broad triplet* signal at δ 5.26 were assigned for the signals of methylene protons H-1" and an olefinic proton H-2", respectively. A *broad triplet* signal at δ 5.03 and two multiplets at δ 1.97-2.00 and 2.02-2.07 were the signals of an olefinic proton H-6" and two groups of methylene proton H-4" and H-5", respectively. There *singlet* signals at δ 1.84, 1.59 and 1.54 were those of three methyl groups. Since the chemical shift of methylene protons H-1" of geranyl side chain was appeared at the lower field than H-1' of isoprenyl side chain, the geranyl group thus was placed nearly the carbonyl group and the isoprenyl moiety was placed at C-2.

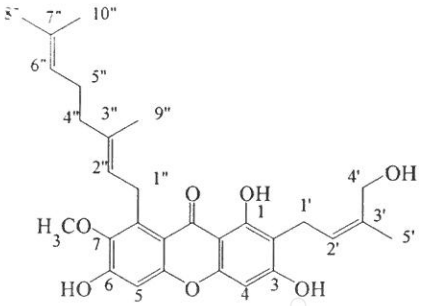
Therefore **3** was assigned to be 1,3,6-trihydroxy-7-methoxy-2-(3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)xanthone. The proposed structure, the spectral data and melting point were agreed with the structure of cowanin (Na Pattalung, *et al.*, 1994).

Table 7 The ^{1}H NMR spectral data of **3**

Position	**3** δ_H (multiplicity, J_{HZ})	[A] **cowanin** δ_H (multiplicity, J_{HZ})
1	13.70 (*s*, OH)	13.80 (*s*, OH)
4	6.30 (*s*, 1H)	6.30 (*s*, 1H)
5	6.83 (*s*, 1H)	6.86 (*s*, 1H)
7-OCH_3	3.80 (*s*, 3H)	3.80 (*s*, 3H)
1'	3.45 (*br d*, 7.0 Hz, 2H)	3.45 (*br d*, 7.0 Hz, 2H)
2'	5.29 (*br t*, 7.0 Hz, 1H)	5.28 (*br t*, 7.0 Hz, 1H)
4'	1.77 (*s*, 3H)	1.76 (*s*, 3H)
5'	1.82 (*s*, 3H)	1.82 (*s*, 3H)
1"	4.10 (*br d*, 7.0 Hz, 2H)	4.09 (*br d*, 7.0 Hz, 2H)
2"	5.26 (*br t*, 7.0 Hz, 1H)	5.28 (*br t*, 7.0 Hz, 1H)
4"	1.97-2.00 (*m*, 2H)	2.03 (*m*, 2H)
5"	2.02-2.07 (*m*, 2H)	2.03 (*m*, 2H)
6"	5.03 (*br t*, 6.5 Hz, 1H)	5.03 (*br t*, 1H)
8"	1.54 (*s*, 3H)	1.59 (*s*, 3H)
9"	1.84 (*s*, 3H)	1.84 (*s*, 3H)
10"	1.59 (*s*, 3H)	1.61 (*s*, 3H)

[A] 400 MHz, in $CDCl_3$

4: Cowanol



Compound **4** is a pale-yellow solid, m.p. 123-124 °C. The UV spectrum showed maximum absorption bands at 355, 316, 255, 244 and 212 nm. The IR spectrum showed the sharp absorption band of conjugated carbonyl group at 1646 cm^{-1} and the broad band of hydroxyl group at 3365 cm^{-1}. The 1H NMR spectrum (**Table 8**) revealed a signal for hydrogen bonded hydroxyl function at δ 13.83. In the aromatic region, two *singlet* resonances at δ 6.85 and 6.32 were assigned for the resonance of H-5 and H-4, respectively. A sharp *singlet* signal at δ 3.82 due to the signal of the proton of methoxy group at C-7. The resonance of geranyl side chain was present in the spectrum. In comparison to the spectrum of compound 2 and 3, the signals of geranyl side chain were assigned as follow; a *doublet* at δ 4.12 and a *doublet of triplet* at δ 5.28 were the signals of methylene protons H-1" and an olefinic proton H-2", a *broad triplet* at δ 5.04 and two sets of *multiplet* signals at δ 2.00-2.03 and 2.04-2.08 belonged to an olefinic proton H-6" and two groups of methylene protons H-4" and H-5", respectively and three *singlets* at δ 1.84, 1.61, and 1.56 were the resonances of three methyl groups. The remaining resonances appearing as a *doublet of triplet* at δ 5.48, a *doublet* at δ 3.54, a singlet with two protons at δ 4.37 and a singlet at δ 1.79 were assigned for olefinic proton H-2′, benzylic methylene protons H-1′, oxymethylene protons H-4′ and methyl protons H-5′, respectively. The evidence (Table 9) suggested that the structure contained the 4-hydroxy-3-methyl-2-butenyl as a side chain. Since the chemical shift of the methylene protons of geranyl

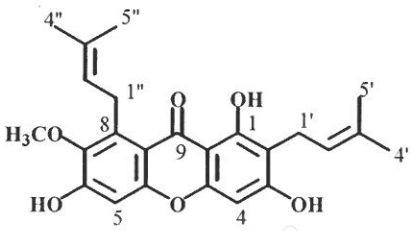
side chain (H-1', δ 3.62) was at the lower field than the methylene protons (H-1', δ 3.49) of 4-hydroxy-3-methyl-2-butenyl side chain, thus the geranyl was proposed to be nearby the carbonyl group and the 4-hydroxy-3-methyl-2-butenyl side chain was located at C-2. The proposed structure, the spectral data and melting of **4** was corresponded to 1,3,6-trihydroxy-7-methoxy-2-(3-methyl-4-hydroxy-2-buteny)-8-(3,7-dimethyl-2,6-octadieny)xanthone or cowanol (Na Pattalung, *et al.*, 1994).

Table 8 The ^{1}H NMR spectral data of **4**

Position	**4** δ_H (multiplicity, J_{HZ})	[A] **cowanol** δ_H (multiplicity, J_{HZ})
1	13.83 (*s*, OH)	13.96 (*s*, OH)
4	6.32 (*s*, 1H)	6.28 (*s*, 1H)
5	6.85 (*s*, 1H)	6.80 (*s*, 1H)
7-OCH_3	3.82 (*s*, 3H)	3.79 (*s*, 3H)
1'	3.54 (*br d*, 7.0 Hz, 2H)	3.51 (*br d*, 2H, 7.0 Hz)
2'	5.48 (*dt*, 7.0,1.5 Hz, 1H)	5.47 (*br t*, 1H , 7.0 Hz)
4'	4.37 (*s*, 2H)	4.35 (*br s*, 2H)
5'	1.79 (*s*, 3H)	1.79 (*s*, 3H)
1"	4.12 (*br d*, 7.0 Hz, 2H)	4.09 (*br d*, 7.0 Hz, 2H)
2"	5.28 (*dt*, 7.0, 1.5 Hz, 1H)	5.24 (*br t*, 7.0 Hz, 1H)
4"	2.00-2.03 (*m*, 2H)	2.03 (*m*, 2H)
5"	2.04-2.08 (*m*, 2H)	2.03 (*m*, 2H)
6"	5.04 (*br t*, 7.0 Hz, 1H)	5.02 (*br t*, 7.0 Hz, 1H)
8"	1.56 (*s*, 3H)	1.54 (*s*, 3H)
9"	1.84 (*s*, 3H)	1.82 (*s*, 3H)
10"	1.61 (*s*, 3H)	1.59 (*s*, 3H)

[A] 400 MHz, in $CDCl_3$

5: 1,3,6-Trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)xanthone (mangostin)



Compound **5** was isolated as a yellow solid, m.p. 182-183 °C. The ^{1}H NMR (**Table 9**) revealed the presence of four sharp *singlet* signals at δ 13.80 (1-OH), 6.82 (H-5), 6.29 (H-4) and 3.81 (7-OCH_3). The signals of two prenyl side chains were observed. Those signals were two *broad triplet* signals of two olefinic protons (δ 5.29 and 5.27), two *doublet* signals of benzylic methylene protons (δ 4.06 and 3.46) and three *singlet* signals of four methyl groups (δ 1.85 (6H), 1.77 and 1.70). Compound **5** was then identified to be 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)xanthone. The ^{1}H NMR spectral data and melting point were identical with mangostin, authentic sample, which was previously isolated from *Garcinia mangostana* (Mahabusarakam, *et al.*, 1987).

Table 9 The ^{1}H NMR spectral data of **5**

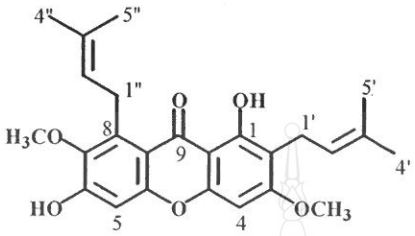
δ_H (*multiplicity*, J_{Hz})	
5*	mangostin**
13.80 (1H, *s*)	13.80 (*s*)
6.82 (1H, *s*)	6.30 (*s*)
6.29 (1H, *s*)	6.28 (*s*)
5.29 (1H, *br t*)	5.28 (*t*, 7.0)
5.27 (1H, *br t*)	5.28 (*br s*, 7.0)
4.06 (2H, *br d*, 5.7)	4.11 (*d*, 7.0)
3.81 (3H, *s*)	3.81 (*s*)
3.46 (2H, *br d*, 6.9)	3.47-3.40 (*d*, 7.0)
1.85 (6H, *s*)	1.84 (*s*), 1.85 (*s*)
1.77 (3H, *s*)	1.78 (*s*)
1.70 (3H, *s*)	1.70 (*s*)

* 300 MHz in $CDCl_3$

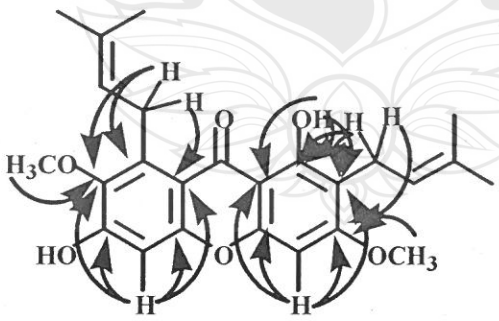
** 400 MHz in $CDCl_3$

6: 1.6-Dihydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)xanthone

(β-mangostin)



Compound **6** is a yellow solid, m.p. 179-180 °C. The ^{1}H NMR spectrum (**Table 10**) indicated the presence of a chelated hydroxy group (1-OH) at δ 13.44, a non chelated hydroxy group (6-OH) at δ 6.40, two isolated aromatic protons (H-4 and H-5) at δ 6.35 and 6.85, two methoxy groups (3-OCH_3 and 7-OCH_3) at δ 3.91 and 3.82, and two prenylated units. The resonances of two prenylated units were shown as followed: four methyl groups at δ 1.69 (*s*, H-4′), 1.80 (*s*, H-5′), 1.70 (*s*, H-4″) and 1.83 (*s*, H-5″), two olefinic methine protons at δ 5.24 (*br t*, H-2′) and 5.27 (*br t*, H-2″) and two benzylic methylene protons at δ 3.36 (*d*, H-1′) and 4.10 (*d*, H-1″). The HMBC correlations confirmed that **6** is 1,6-dihydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)xanthone or known as β-mangostin (Yates and Bhat, 1968).



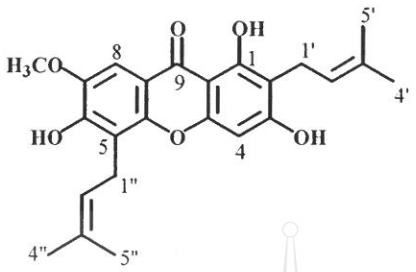
Major HMBC correlations of **6**

Table 10 The NMR spectral data of **6**

Position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	HMBC
1	159.76 (C)	-	-
2	111.51 (C)	-	-
3	163.52 (C)	-	-
4	88.81 (CH)	6.35 (1H, *s*)	C-2, C-3, C-4a, C-9, C-9a
4a	155.71 (C)	-	-
5	101.48 (CH)	6.85 (1H, *s*)	C-6, C-7, C-8a, C-9, C-10a
6	154.44 (C)	-	-
7	142.57 (C)	-	-
8	137.03 (C)	-	-
8a	112.38 (C)	-	-
9	181.98 (C=O)	-	-
9a	101.13 (C)	-	-
10a	155.24 (C)	-	-
1′	21.35 (CH_2)	3.36 (2H, *d*, 7.0)	C-1, C-2, C-3, C-2′, C-3′
2′	122.31 (CH)	5.24 (1H, *br t*, 7.0)	C-1′, C-4′, C-5′
3′	131.72 (C)	-	-
4′	25.83 (CH_3)	1.69 (3H, *s*)	C-2′, C-3′, C-5′
5′	17.79 (CH_3)	1.80 (3H, *s*)	C-2′, C-3′, C-4′
1″	26.55 (CH_2)	4.10 (2H, *d*, 6.5)	C-7, C-8, C-8a, C-2″, C-3″
2″	123.20 (CH)	5.27 (1H, *br t*, 6.5)	C-1″, C-4″, C-5″
3″	132.10 (C)	-	-
4″	25.84 (CH_3)	1.70 (3H, *s*)	C-2″, C-3″, C-5″
5″	18.23 (CH_3)	1.83 (3H, *s*)	C-2″, C-3″, C-4″
1-OH	-	13.44 (1H, *s*)	C-1, C-2, C-9a
6-OH	-	6.40 (1H, *s*)	-
3-OCH_3	55.84 (CH_3)	3.91 (3H, *s*)	C-3
7-OCH_3	56.05 (CH_3)	3.82 (3H, *s*)	C-7

* Carbon type was deduced from DEPT experiments.

7: 1,3,6-trihydroxy-7-methoxy-2,5-bis (3-methyl-2-butenyl)xanthone

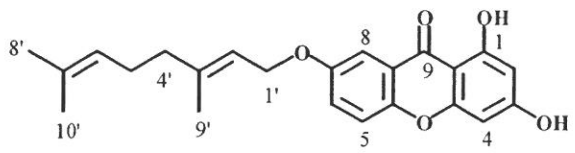


Compound **7** is a bright yellow solid, m.p. 223-224 °C. The ^{1}H NMR spectrum (**Table 11**) exhibited the resonances of hydroxy protons 1-OH at δ 13.22 (*s*), 3-OH at δ 8.99 (*s*) and 6-OH at δ 7.11 (*s*), aromatic protons H-8 at δ 7.44 (*s*) and H-4 at δ 6.37 (*s*) and 7-OCH_3 at δ 3.96 (*s*). The NOE effect of H-8 (δ 7.44) to the methoxy resonance suggested the OCH_3 at C-7. The signals of two prenyl groups were displayed as followed: two *broad triplets* of olefinic methine protons H-2′ (δ 5.26) and H-2″ (δ5.25), two *doublets* of benzylic methylene protons H-1′ (δ 3.38) and H-1″ (δ 3.56) and four *singlets* of methyl groups H-4′ (δ 1.69), H-5′ (δ 1.80), H-4″ (δ 1.65) and H-5″ (δ 1.85). The HMBC correlations of H-1′ to C-1, C-2, C-3 and H-1″ to C-5, C-6, C-10a indicated that the prenyl side chains were at C-2 and C-5. The assignment of H-4 was confirmed by the correlation of H-4 to C-2, C-3, C-4a, C-9 and C-9a whereas the signal of H-8 was proved by the correlation of H-8 to C-6, C-7, C-8a, C-9 and C-10a. These assignment indicated that **7** was 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone. The structure of **7** and its melting point were identical to the previously isolated compound from *Garcinia cowa* (Na Pattalung, *et al.*, 1994).

Table 11 The NMR spectral data of **7**

Position	δ_C (C-Type)	δ_H (*multiplicity*, J_{Hz})	HMBC
1	160.00 (C)	-	-
2	109.65 (C)	-	-
3	160.12 (C)	-	-
4	93.55 (CH)	6.37 (1H, *s*)	C-2, C-3, C-4a, C-9, C-9a
4a	155.86 (C)	-	-
5	115.30 (C)	-	-
6	150.05 (C)	-	-
7	144.05 (C)	-	-
8	101.99 (CH)	7.44 (1H, *s*)	C-6, C-7, C-8a, C-9, C-10a
8a	112.94 (C)	-	-
9	180.21 (C=O)	-	-
9a	101.99 (C)	-	-
10a	149.50 (C)	-	-
1′	21.36 (CH_2)	3.38 (2H, *d*, 6.9)	C-1, C-2, C-3, C-2′, C-3′
2′	121.90 (CH)	5.26 (1H, *br t*, 7.2)	C-1′, C-4′, C-5′
3′	133.60 (C)	-	-
4′	25.70 (CH_3)	1.69 (3H, *s*)	C-2′, C-3′, C-5′
5′	17.83 (CH_3)	1.80 (3H, *s*)	C-2′, C-3′, C-4′
1″	22.34 (CH_2)	3.56 (2H, *d*, 7.2)	C-5, C-6, C-10a, C-2″, C-3″
2″	121.03 (CH)	5.25 (1H, *br t*, 7.2)	C-1″, C-4″, C-5″
3″	132.57 (C)	-	-
4″	25.75 (CH_3)	1.65 (3H, *s*)	C-2″, C-3″, C-5″
5″	17.90 (CH_3)	1.85 (3H, *s*)	C-2″, C-3″, C-4″
1-OH	-	13.22 (1H, *s*)	C-1, C-2, C-9a
3-OH	-	8.99 (1H, *s*)	-
6-OH	-	7.11 (1H, *s*)	-
7-OCH_3	56.33 (CH_3)	3.96 (3H, *s*)	C-7

8: 7-Geranyloxy-1,3-dihydroxyxanthone

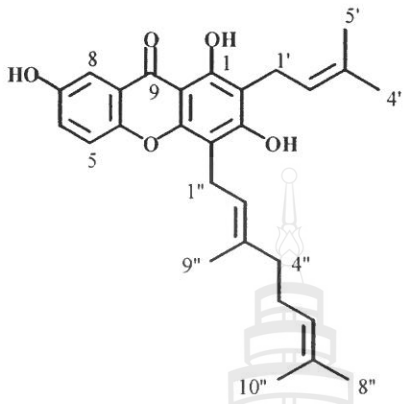


Compound **8** is a yellow solid, m.p. 137-138 °C. The UV spectrum showed maximum absorption bands at 204, 232, 259, 310 and 368 nm. The IR spectrum showed the absorption band of a conjugated carbonyl group at 1650 cm^{-1} and a hydroxyl group at 3137 cm^{-1}. The ^{1}H NMR spectral data (**Table 12**) revealed the presence of a chelated hydroxyl proton (1-OH) at δ 12.70 (*s*) and *meta* protons H-2 and H-4 at δ 6.29 (*d*, J = 3.0 Hz) and 6.38 (*d*, J = 3.0 Hz). The spectrum further showed the signals of ABX system of H-5, H-6 and H-8 at δ 7.35 (*d*, J = 9.0 Hz), 7.32 (*dd*, J = 9.0, 3.0 Hz) and 7.61 (*d*, J = 3.0 Hz), respectively. The geranyl unit was commemorated from distinctive signals of two olefinic protons at δ 5.50 (H-2′, J = 6.6 Hz) and 5.09 (H-6′, J = 6.6 Hz), three methylene protons at δ 4.61 (*d*, J = 6.6 Hz, H-1′) and 2.12 (*m*, H-4′ and H-5′) and three vinyl methyl protons at δ 1.60 (*s*, H-8′), 1.76 (*s*, H-9′) and 1.67 (*s*, H-10′). It was assigned as an *O*-geranyl unit according to the chemical shift of H-1′ (δ 4.61). The differential NOE technique by irradiation of the signal of H-1′ affected the signal of H-8, accordingly *O*-geranyl unit was assigned at C-7. The correlation of H-1′ to C-7 in HMBC experiment comfirmed the position of a geranyl unit at C-7. Compound **8** was denoted as 7-geranyloxy-1,3-dihydroxyxanthone (Nguyen, L.H.D. and Harrison, L.J., 1998).

Table 12 The NMR spectral data of **8**

Position	δ_C (C-Type)	δ_H (*multiplicity*, J_{Hz})	HMBC
1	163.5 (C)	-	-
2	98.3 (CH)	6.29 (1H, *d*, 3.0)	C-1, C-4
3	157.9 (C)	-	-
4	94.1 (CH)	6.38 (1H, *d*, 3.0)	C-2, C-3, C-9a
4a	142.0 (C)	-	-
5	118.8 (CH)	7.35 (1H, *d*, 9.0)	C-6, C-7, C-8a
6	125.2 (CH)	7.32 (1H, *dd*, 9.0, 3.0)	C-10a
7	155.3 (C)	-	-
8	106.2 (CH)	7.61 (1H, *d*, 3.0)	C-5, C-6, C-7, C-9, C-10a
8a	120.6 (C)	-	-
9	180.6 (C=O)	-	-
9a	103.6 (C)	-	-
10a	150.7 (C)	-	-
1′	65.6 (CH_2)	4.61 (2H, *d*, 6.6)	C-7, C-2′, C-3′
2′	118.7 (CH)	5.50 (1H, *t*, 6.6)	C-9a, C-4′
3′	142.0 (C)	-	-
4′	39.5 (CH_2)	2.12 (2H, *m*)	C-2′, C-3′, C-5′
5′	26.3 (CH_2)	2.12 (2H, *m*)	C-3′, C-4′, C-6′
6′	123.7 (CH)	5.09 (1H, *t*, 6.6)	C-5′, C-10′
7′	131.8 (C)	-	-
8′	17.6 (CH_3)	1.60 (3H, *s*)	C-6′, C-7′, C-10′
9′	16.7 (CH_3)	1.76 (3H, *s*)	C-2′, C-3′, C-4′
10′	25.6 (CH_3)	1.67 (3H, *s*)	C-6′, C-7′, C-8′
1-OH	-	12.96 (1H, *s*)	C-2, C-9

9: 1,3,7-Trihydroxy-2-(3-methyl-2-butenyl)-4-(3,7-dimethyl-2,6-octadienyl)xanthone (Cochinchinone A)



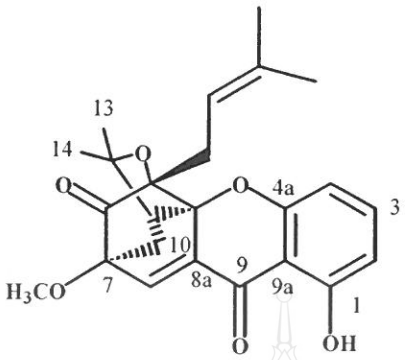
Compound **9** is a yellow solid, m.p. 119-120 °C. The UV spectrum showed maximum absorption at 232, 268, 316 and 384 nm. The IR spectrum showed the absorption band of a conjugated carbonyl group at 1641 cm^{-1} and a hydroxyl group at 3413 cm^{-1}. The ^1H NMR spectrum (**Table 13**) showed a *singlet* signal of a deshielded proton 1-OH at δ 12.96. The ABX system in the aromatic region, δ 7.54 (*d*, J = 3.0 Hz), 7.36 (*d*, J = 9.0 Hz) and 7.24 (*dd*, J = 9.0, 3.0 Hz) were assigned for H-8, H-5 and H-6, respectively. The presence of characteristic signals of a prenyl unit, were shown at δ 3.37 (*d*, J = 7.0 Hz, H-1′), 5.20 (*br t*, J = 7.0 Hz, H-2′), 1.79 (*s*, H-4′) and 1.69 (*s*, H-5′). The location of a prenyl group at C-2 was supported by HMBC correlation of H-1′ to C-1, C-2 and C-3. The remaining signals revealed the presence of a geranyl group. A *doublet* signal at δ 3.48 and a *broad triplet* signal at δ 5.20 were assigned for the signals of methylene protons H-1″ and an olefinic proton H-2″, respectively. A *broad triplet* signal at δ 5.00 and *multiplet* at δ 1.93-2.10 were the signals of an olefinic proton H-6″ and methylene protons H-4″ and H-5″, respectively. Three *singlet* signals at δ 1.50, 1.83 and 1.60 were those of three methyl groups. The geranyl side chain was located at C-4 which was confirmed by the cross

peak of H-1'' to C-3 and C-4a in HMBC correlation. The assignment then suggested that compound **9** is cochinchinone A (Mahabusarakam, *et al.*, 2006).

Table 13 The ^{1}H NMR spectral data of **9**

Position	**9** δ_H (multiplicity, J_{HZ})	**cochinchinone A** δ_H (multiplicity, J_{HZ})
5	7.36 (1H, *d*, 9.0)	7.36 (1H, *d*, 9.0)
6	7.24 (1H, *dd*, 9.0, 3.0)	7.24 (1H, *dd*, 9.0, 3.0)
8	7.54 (1H, *d*, 3.0)	7.59 (1H, *d*, 3.0)
1'	3.37 (2H, *d*, 7.0)	3.47 (2H, *d*, 7.0)
2'	5.20 (1H, *br t*, 7.0)	5.29 (1H, *br t*, 7.0)
4'	1.79 (3H, *s*)	1.84 (3H, *s*)
5'	1.69 (3H, *s*)	1.76 (3H, *s*)
1"	3.48 (2H, *d*, 7.0)	3.57 (2H, *d*, 7.0)
2"	5.20 (1H, *br t*, 7.0)	5.27 (1H, *br t*, 7.0)
4"	1.93-2.10 (2H, *m*)	2.03-2.06 (2H, *m*)
5"	2.11-1.93 (2H, *m*)	2.11-2.08 (2H, *m*)
6"	5.00 (1H, *br t*, 7.0)	5.05 (1H, *br t*, 7.0)
8"	1.50 (3H, *s*)	1.57 (3H, *s*)
9"	1.83 (3H, *s*)	1.88 (3H, *s*)
10"	1.60 (3H, *s*)	1.64 (3H, *s*)
1-OH	12.96 (1H, *s*)	12.95 (1H, *s*)

10: Cratoxycochinchinone C

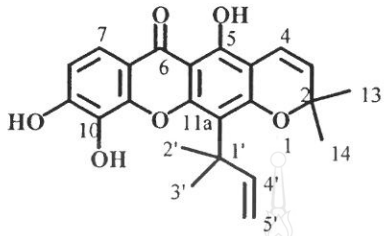


Compound **10** is a yellow solid, m.p. 147-148 °C, $[\alpha]_D^{29}$ = + 98° (c 1.0x10^{-2} g/cm^{-3} in $CHCl_3$). The ^{1}H NMR spectrum (**Table 14**) showed a *singlet* signal of a deshielded proton 1-OH at δ 12.00. The signal at δ 3.64 belonged to a methoxyl group which was assigned at C-7. The ABX pattern in the aromatic proton region, δ 6.54 (*dd*, *J* = 8.4 and 1.0 Hz), 7.41 (*t*, *J* = 8.4 Hz) and 6.51 (*dd*, *J* = 8.4 and 1.0 Hz) corresponded to H-2, H-3 and H-4, respectively. The singlet resonance of olefinic proton H-8 (δ 7.51) and two *doublet of doublet* signals (δ 1.59 and 2.38) of non-equivalent methylene protons H_b-10 and H_a-10 revealed the quarternary carbon at C-7. The presence of a prenyl unit was identified from two *singlet* signals of *gem*-dimethyl protons H-18 and H-19 at δ 1.37 and 1.01, a *broad triplet* of olefinic proton H-16 at δ 4.40 and a *doublet* of allylic methylene protons 2H-15 at δ 2.64. Its physical and spectral data were in agreement with those of cratoxycochinchinone C (Mahabusarakam, *et al.*, 2006).

Table 14 The ^{1}H NMR spectral data of **10**

Position	**10** δ_H (multiplicity, J_{HZ})	**cratoxycochinchinone C** δ_H (multiplicity, J_{HZ})
2	6.54 (1H, *dd*, 8.4, 1.0)	6.55 (1H, *dd*, 8.4, 0.9)
3	7.41 (1H, *t*, 8.4)	7.41 (1H, *t*, 8.4)
4	6.51 (1H, *dd*, 8.4, 1.0)	6.52 (1H, *dd*, 8.4, 0.9)
8	7.51 (1H, *s*)	7.51 (1H, *s*)
10a	2.38 (1H:H_a-10, *d*, 12.9)	2.39 (1H:H_a-10, *d*, 12.9)
	1.59 (1H:H_b-10, *dd*, 12.9, 9.9)	1.59 (1H:H_b-10, *dd*, 12.9, 9.9)
13	1.68 (3H, *s*)	1.69 (3H, *s*)
14	1.32 (3H, *s*)	1.33 (3H, *s*)
15	2.64 (2H, *d*, 8.1)	2.64 (2H, *d*, 8.1)
16	4.40 (1H, *br t*, 8.1)	4.39 (1H, *br t*, 8.1)
18	1.37 (3H, *s*)	1.37 (3H, *s*)
19	1.01 (3H, *s*)	1.01 (3H, *s*)
1-OH	12.00 (1H, *s*)	12.00 (1H, *s*)
7-OCH_3	3.64 (3H, *s*)	3.65 (3H, *s*)

11: 5,9,10-Trihydroxy-12-(1,1-dimethyl-2-propenyl)-2*H*,6*H*-pyrano[3,2-*b*] xanthen-6-one (Macluraxanthone)

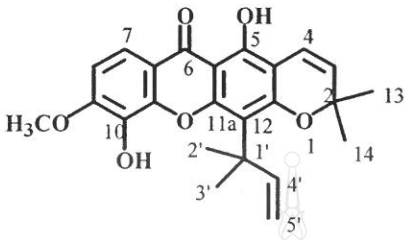


Compound **11** is a yellow solid, m.p. 170-172 °C. The ^{1}H NMR spectrum exhibited a *singlet* signal of hydrogen bonded hydroxyl function (5-OH) at δ 13.56. Two *doublet* signals at δ 7.71 and 6.97 were observed and assigned to be the signals of aromatic proton H-7 and H-8. The ^{1}H NMR spectrum (**Table 15**) revealed a characteristic signal of a dimethylchromene ring, of which the signal of *gem*-dimethyl protons resonated as a *singlet* at δ 1.53 and two *doublet* signals of two *cis*-olefinic protons (H-4 and H-3) were at δ 6.80 and 5.64. The signals of *gem*-dimethyl protons (H-2′ and H-3′) at δ 1.67, a *cis*-olefinic protons at δ 6.76 (H-4′) and terminal olefinic methylene protons at δ 5.24 (H-5′ *Z*) and 5.07 (H-5′ *E*) corresponded to 1,1-dimethyl-2-propenyl substituent. The ^{13}C NMR spectrum and DEPT experiments indicated the presence of a carbonyl carbon (δ 180.79), four methyl carbons (δ 28.20x2 and 27.94x2), a methylene carbon (δ 103.29), five methine carbons (δ 156.90, 127.16, 117.53, 116.14 and 112.76) and twelve quaternary carbons (δ 158.94, 156.79, 154.11, 149.01, 144.55, 131.05, 113.74, 113.07, 105.59, 103.08, 78.26 and 41.46). The proposed structure, the spectral data and melting point agreed with 5,9,10-trihydroxy-12-(1,1-dimethyl-2-propenyl)-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one or macluraxanthone (Iinuma, *et al.*, 1994).

Table 15 The ^{1}H NMR spectral data of **11**

Position	**11** δ_H (multiplicity, J_{HZ})	**cratoxycochinchinone C** δ_H (multiplicity, J_{HZ})
3	5.64 (1H, *d*, 10.2)	5.70 (1H, *d*, 10.0)
4	6.80 (1H, *d*, 10.2)	6.69 (1H, *d*, 10.0)
5-OH	13.56 (1H, *s*)	13.91 (1H, *s*)
7	7.71 (1H, *d*, 9.0)	7.60 (1H, *d*, 9.0)
8	6.97 (1H, *d*, 9.0)	7.00 (1H, *d*, 9.0)
13	1.53 (3H, *s*)	1.49 (3H, *s*)
14	1.53 (3H, *s*)	1.49 (3H, *s*)
2′	1.67 (3H, *s*)	1.74 (3H, *s*)
3′	1.67 (3H, *s*)	1.74 (3H, *s*)
4′	6.76 (1H, *dd*, 17.7, 10.8)	6.52 (1H, *dd*, 17.0, 11.0)
5′ *Z*	5.24 (1H, *dd*, 17.7, 1.2)	5.05 (1H, *dd*, 17.0, 1.0)
5′ *E*	5.07 (1H, *dd*, 10.8, 1.2)	4.89 (1H, *dd*, 11.0, 1.0)

12: 5,10-Dihydroxy-9-methoxy-12-(1,1-dimethyl-2-propenyl)-2*H*,6*H*-pyrano [3,2-*b*]xanthen-6-one (10-*O*-Methylmacluraxanthone)



Compound **12** is a yellow solid. The ^{1}H NMR spectrum (**Table 16**) exhibited a *singlet* signals of hydrogen bonded hydroxyl function (5-OH) at δ 13.48. The *ortho* coupling pattern in aromatic region, δ 7.68 (*d*) and 6.97 (*d*) were proposed for the characteristic signals of H-7 and H-8, respectively. The most deshielded aromatic proton signal was assigned for H-7 according to anisotropic effect of the carbonyl group. Two sharp *singlet* signals were due to the signal of 9-OCH_3 (δ 3.95) and 10-OH (δ 6.16). The typical signal of an isoprenyl unit was observed. The signals of two *singlets* of *gem*-dimethyl protons H-2′ and H-3′ (δ 1.59), two *doublets* of terminal olefinic methylene protons H-5′*E* (δ 5.11) and H-5′*Z* (δ 4.98) and a methane proton resonating at δ 6.59 were assigned for the isoprenyl unit at C-12 according to the correlation of the proton H-4′ to C-12 in the HMBC spectrum. The remaining signals were assigned for a chromene ring. Two vicinal protons H-3 and H-4 appeared as two *doublet* signals at δ 5.44 and 6.70. The correlations of H-4 to C-4a, C-5 and H-3 to C-4a, C-13, C-14 correctly determined that the dimethyl chromene ring was next to C04a and C-12a. The ^{13}C NMR spectrum and DEPT experiments indicated the presence of a carbonyl carbon (δ 181.00), five methyl carbons (δ 56.59, 28.50x2 and 27.93x2), a methylene carbon (δ 104.64), five methine carbons (δ 155.02, 127.18, 116.82, 116.06 and 108.43) and twelve quaternary carbons (δ 159.14, 156.71, 154.50, 151.50, 144.39, 133.53, 114.30, 113.35, 105.43, 103.11, 78.26 and 41.34). Compound

12 was identified as 5,10-dihydroxy-9-methoxy-12-(1,1-dimethyl-2-propenyl)-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one or 10-*O*-Methylmacluraxanthone (Boonnak, *et al.*, 2006).

Table 16 The NMR spectral data of **12**

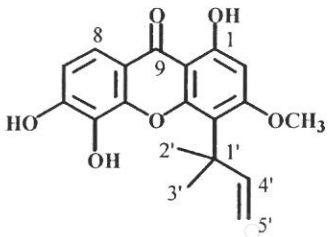
Position	δ_C (C-Type)	δ_H (*multiplicity*, J_{Hz})	HMBC
2	78.26 (C)	-	-
3	127.18 (CH)	5.54 (1H, *d*, 10.2)	C-2, C-4a, C-13, C-14
4	116.06 (CH)	6.70 (1H, *d*, 10.2)	C-2, C-4a, C-5, C-12a
4a	105.43 (C)	-	-
5	156.71 (C)	-	-
5a	103.11 (C)	-	-
6	181.00 (C=O)	-	-
6a	114.30 (C)	-	-
7	116.82 (CH)	7.68 (1H, *d*, 9.0)	C-6, C-9, C-10a
8	108.43 (CH)	6.90 (1H, *d*, 9.0)	C-6a, C-7, C-9, C-10
9	151.50 (C)	-	-
10	133.53 (C)	-	-
10a	144.39 (C)	-	-
11a	154.50 (C)	-	-
12	113.35 (C)	-	-
12a	159.14 (C)	-	-
13	27.93 (CH_3)	1.44 (3H, *s*)	C-3, C-14
14	27.93 (CH_3)	1.44 (3H, *s*)	C-2, C-13
1′	41.34 (C)	-	-
2′	28.50 (CH_3)	1.59 (3H, *s*)	C-12, C-1′, C-3′
3′	28.50 (CH_3)	1.59 (3H, *s*)	C-12, C-1′, C-2′
4′	155.02 (CH)	6.59 (1H, *dd*, 17.7, 10.8)	C-12, C-1′, C-2′, C-3′

Table 16 (continued)

Position	δ_C (C-Type)	δ_H (*multiplicity*, J_{Hz})	HMBC
5′*E*	104.64 (CH)	5.11 (1H, *d*, 17.7)	C-1′, C-4′
5′*Z*	104.64 (CH)	4.98 (1H, *d*, 10.8)	C-1′
5-OH	-	13.35 (1H, *s*)	C-4a, C-5, C-5a
10-OH	-	6.16 (1H, *s*)	C-9, C-10, C-10a
9-OCH_3	56.59 (CH_3)	3.95 (3H, *s*)	C-9

13: 1,5,6-Trihydroxy-3-methoxy-4-(1,1-dimethyl-2-propenyl)xanthone

(Isocudraniaxanthone B)



Compound **13** is a yellow solid, m.p. 226-228 °C. The UV spectrum showed maximum absorption bands at 204, 237, 253, 287 and 328 nm. The IR spectrum showed the absorption band of a conjugated carbonyl group at 1647 cm^{-1} and a hydroxyl group at 3417 cm^{-1}.The ^1H NMR spectrum (**Table 17**) showed signals for a hydrogen-bonded hydroxyl group at δ 13.35 (*s*), an aromatic proton H-2 at δ 6.40 (*s*) and two *ortho* aromatic protons H-7 (δ 6.95, *d*, J = 8.7 Hz) and H-8 (δ 7.69, *d*, J = 8.7 Hz). H-8 was assigned to a low field chemical shift and from HMBC correlations of H-8 to C-6 and C-9; H-7 to C-5, C-6 and C-8a. The signal of two *singlets* of *gem*-dimethyl protons at δ 1.61 (H-2′) and δ 1.61 (H-3′), an olefinic proton at δ 6.70 (H-4′) and terminal olefinic methylene protons at δ 5.03 (H-5′*Z*) and δ 5.21 (H-5′*E*) were characteristic signals of an isoprenyl unit. Compound **13** was indicated as isocudraniaxanthone B (Hay, *et al.*, 2004).

Table 17 The NMR spectral data of **13**

Position	δ_C (C-Type)	δ_H (*multiplicity*, J_{Hz})	HMBC
1	162.4 (C)	-	-
2	95.6 (CH)	6.40 (1H, *s*)	C-1, C-2, C-3
3-OCH_3	55.7 (CH_3)	3.89 (3H, *s*)	C-3
4	113.0 (C)	-	-
4a	154.0 (C)	-	-
5	131.0 (C)	-	-
6	149.1 (C)	-	-
7	112.6 (CH)	6.95 (1H, *d*, 8.7)	C-5, C-6, C-8a
8	117.5 (CH)	7.69 (1H, *d*, 8.7)	C-6, C-9
8a	113.0 (C)	-	-
9	180.8 (C=O)	-	-
9a	103.0 (C)	-	-
10a	(C)	-	-
1′	41.5 (C)	-	-
2′	27.8 (CH_3)	1.61 (3H, *s*)	C-4, C-1′, C-4′
3′	27.8 (CH_3)	1.61 (3H, *s*)	C-4, C-1′, C-4′
4′	156.8 (CH)	6.70 (1H, *dd*, 17.7, 10.5)	C-4, C-1′, C-2′, C-3′
5′ *Z*	103.2 (CH_2)	5.03 (1H, *dd*, 10.5, 1.5)	C-1′
5′ *E*	103.2 (CH_2)	5.09 (1H, *dd*, 17.7, 1.5)	C-1′, C-4′
1-OH	-	13.35 (1H, *s*)	-

4.2 Evaluation of Antioxidation Properties

Recently, natural antioxidants seemed to be very attractive because some synthetic antioxidants have been found to be carcinogenic and harmful to lungs and liver. Phenolic compounds were known to be the antioxidation with an excellent hydrogen or electron donor (Shahidi *et al.*, 1992). Most of components isolate from *G. cowa* were xanthones that contained free phenolic hydroxyl group. It was thus of considerable interest in the studies of antioxidant properties.

Estimation of antioxidative effects has been carried out by various methods. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is one of the methods used for testing of antioxidative properties. DPPH is a stable free radical which shows a purple color and a strong absorption at 517 nm. It has been used as a convenient tool for the antioxidant assay of biological materials. When DPPH radical accepts hydrogen radical, a more stable compound will be formed and consequently its characteristic absorption at 517 nm vanishes. The capacity of the substances to donate electrons can be estimated from the degree of loss of color (Blois, 1958).

Coexistence of an antioxidant compound (AH) and free radical DPPH leads to the disappearance of DPPH free radical and to the appearance the free radical $A^{\bullet}$ (**Figure 4**).

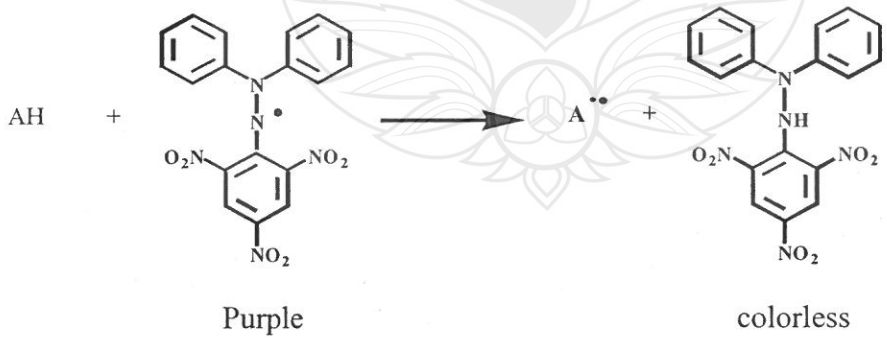


Figure 4 DPPH free radical and the appearance the free radical $A^{\bullet}$

4.2.1 Screening on the free radical scavenging activity of the crude material

To determine the scavenging activity, crude acetone and crude methanolic extracts from the root of *G. cowa* were conducted at the final concentration at 100 µg/mL. The activity was monitored by following the decrease of absorbance of the solution at 517 nm.

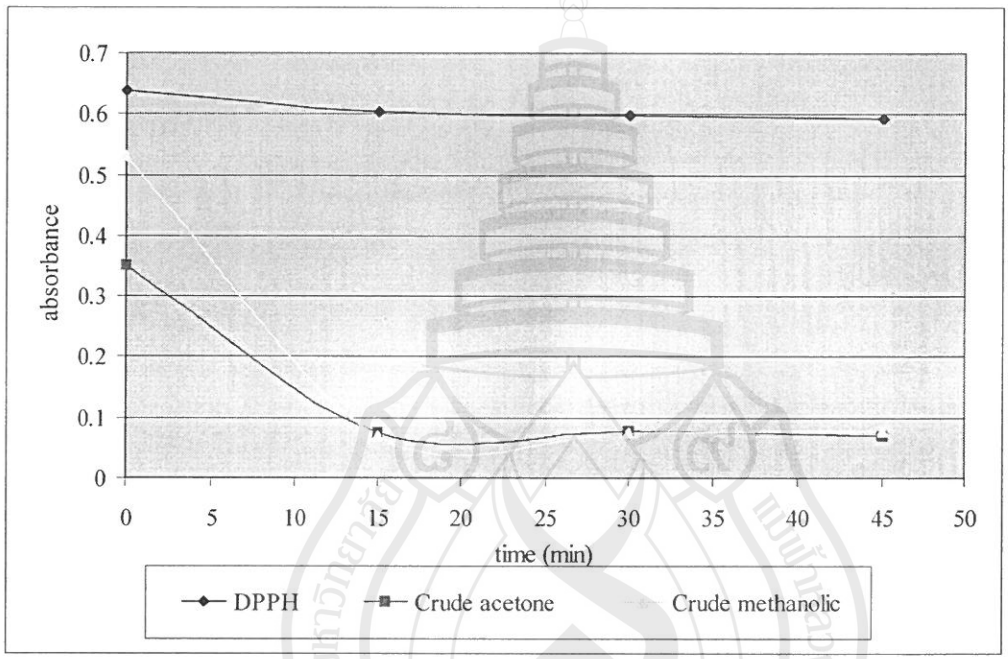


Figure 5 Antioxidation activity of the crude materials against DPPH radical

The results (**Figure 5**) indicated that the crude acetone and crude methanolic extracts were able to scavenge the DPPH radical significantly.

The assessment of the antioxidation activity of the crude materials were extended. In comparable to the standard antioxidants (BHT and ascorbic acid) and crude materials were evaluated for IC_{50}. Since the decolorization occurred properly within 30 min, the IC_{50} then was examined at 30 min.

Table 18 Inhibitory concentration (IC_{50}) of the crude materials comparable to BHT and ascorbic acid (at final concentration 100 µg/mL)

Sample	IC_{50} ± S.D. (µg/mL, 30 min)
crude acetone	11.25 ± 0.02
crude methanolic	17.85 ± 0.02
BHT	15.10 ± 0.01
Ascorbic acid	10.30 ± 0.01

The results (**Table18**) showed that crude acetone and methanolic extracts showed IC_{50} at 11.25 and 17.85 µg/mL, respectively. Whereas BHT and ascorbic acid exhibited IC_{50} at 15.10 and 10.30 µg/mL, respectively.

4.2.2 Free radical scavenging activity of the pure compounds

To determine the active chemical constituent of the root of *G. cowa*, pure constituents were examined for the activity. The final concentrations of tested samples were conducted at final concentration 10 µM. The absorption of the solution of the solution of the tested samples and DPPH were measured at 517 nm at room temperature. The activity was expressed in the percentage inhibition.

Radical scavenging activities of some pure compounds from the root of *G. cowa* were evaluated against the DPPH radical. The results (**Table 19**) suggested that the radical scavenging activity of the crude materials moderate exhibit by **11** and **13**.

Table 19 Inhibitory concentration (IC_{50}) of compound **11** and **13** comparable to BHT and ascorbic acid (at final concentration 10 μM)

Sample	IC_{50} ± S.D. (μM, 30 min)
11	7.40 ± 0.02
13	6.50 ± 0.02
BHT	8.70 ± 0.02
Ascorbic acid	4.30 ± 0.02

CHAPTER 5

CONCLUSION

Isolation of the chemical constituents from the root of *Garcinia cowa* Roxb., by chromatographic and crystallization technique, yielded a new xanthone: 3-geranyloxy-1,7-dihydroxyxanthone (**1**) and twelve previously reported xanthones: cowaxanthone (**2**), cowanin (**3**), cowanol (**4**), mangostin (**5**), *β*-mangostin (**6**), 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone (**7**), 7-geranyloxy-1,3-dihydroxyxanthone (**8**), cochinchinone A (**9**), crytoxycochinchinone C (**10**), macluraxanthone (**11**), 10-*O*-methylmacluraxanthone (**12**), isocudraniaxanthone B (**13**), stigmasterol (**14**). Their structures were elucidated on the basis of 1D and 2D NMR spectroscopic data.

The crude materials (crude acetone and crude methanolic extracts) and its pure compounds **1-13** were examined for their antioxidative activity by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The crude material exhibited strong activity with IC_{50} 11.25 and 17.85 μg/mL for crude acetone and crude methanolic extracts, respectively. Whereas compound **11** and **13** showed strong activity (IC_{50} 7.40 and 6.50 μM, respectively) than that of BHT (IC_{50} 8.70 μM) and the other compounds (**1-10** and **12**) showed weak activity.

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2006	Ph.D.	Organic Chemistry	Prince of Songkla University
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1999	B.Sc.	Chemistry	Prince of Songkla University

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1995-1999 Bachelor of Science in Chemistry. Prince of Songkla University.

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1983	B.Sc.	Chemistry	Prince of Songkla University

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1. Deachathai, S; **Mahabusarakam, W.**; Phongpaichai, S.; Taylor, W.C.; Zhang, Y.-J. and Yang, C.-R. 2005. "Phenolic compounds from the flowers of *Garcinia dulcis*", *Phytochemistry,* 67, 464-469

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