FULL REPORT

Isolation and Structural Elucidation of Bioactive Compounds from Twigs of *Garcinia cowa*

By

Dr. Wong Phakhodee

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EXECUTUVE SUMMARY

Collected and prepared plant material

Collected plant material

The twigs of *G. cowa* were collected from Nongkhai province in Northeast part of Thailand in March 2010, and identified by Mr. James Maxwell, Chiang Mai University Herbarium. A voucher specimen (MFU-NPR 0012) was deposited at Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100 Thailand.

Prepared plant material

The twigs of *G. cowa* were cut down to small pieces and dried over air-dry for a week. This material were extracted with acetone over the period of 3 days at room temperature. Removal of solvent under reduced pressure provided acetone extract.

Isolation

The crude extract was purified by chemical techniques including column chromatography, crystallization to give six known xanthones, cowanin **(1)**, cowanol **(2)**, α-mangostin **(16)**, 1,7-dihydroxyxanthone **(28)**, 1,3,6,7-tetrahydroxyxanthone **(30)**, 1,3,7-trihydroxy-2-prenylxanthone **(50)** and a new depsidone namely cowadepsidone **(51)**.

Structure elucidation

All isolated chemical structure, namely listed above, were determined on the basis of spectroscopic methods containing, FT-IR spectrophotometer ^{1}H and ^{13}C NMR spectrometer MicroTOF mass spectrometer.

Anti-bacterial activity

Almost compounds were further evaluated for their antibacterial activity against Gram negative: *Escherichia coli* TISTR 780, *Salmonellae typhimurium* TISTR 292 and Gram positive: *Staphylococcus aureus* TISTR 1466 (SA) and Methicillin-resistant *S. aureus* (MRSA) SK1. Cowanol (**2**) having geranyl group at C-8 plays an important role in the antibacterial activity against both MRSA SK1 and *S. aureus* with MIC values of 2 and 8 μg/mL, respectively.

PUBLICATION

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A NEW DEPSIDONE FROM THE TWIGS OF *GARCINIA COWA*

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Abstract – A new depsidone, cowadepsidone (7), along with six known xanthones (**1-6**) were isolated from the twigs of *Garcinia cowa*. Their structures were determined on the basis of spectroscopic methods. The cytotoxicity against KB, MCF-7 and NCI-H187 cancer cell lines of compounds **2**-7 were also reported.

The tropical family Clusiaceae is well known to be a rich source of isoprenylated xanthones, depsidones and biflavonoids.[1-4] In particular, the genus *Garcinia* has also provided many bioactive isoprenylated and rearranged xanthonoids and biflavonoids.[3-7] In our continuing phytochemical study of Thai medicinal plants, we have found many polyoxygenated xanthones from the barks and dried fruits of *Cratoxylum cochinchinense*.[8,9] In this paper, we describe the isolation and structural elucidation of a new depsidone together with six known xanthones (Figure 1) as well as their cytotoxic activity.

Compound **7** was isolated as a red gum. Its molecular formula was established as $C_{19}H_{18}O_7$ by ESITOFMS at *m/z* 359.1133 $[M+H]^+$, suggesting the presence of 11 degrees of unsaturations and supported by NMR data (Table 1). The IR spectrum showed absorption bands for hydroxyl group (3363 cm^{-1}) and a lactone carbonyl group chelated to an *ortho*-hydroxyl group (1658 cm^{-1}). The presence of the latter functionality was confirmed by resonances at δ 168.0 (C-11) and δ 10.98 (OH-1). The ^1H NMR data of **7** displayed signals of two aromatic protons at δ 6.70 (1H, s, H-6) and 6.27 (2H, br s, H-2, H-4).

Furthermore, the proton signals at δ 5.17 (1H, br s, H-13), 3.47 (2H, d, J = 6.8 Hz, H_2-12), 1.80 (3H, s, H_3-15), and 1.67 (3H, s, H_3-16) suggested the presence of a prenyl moiety in the structure.[9] In addition, a methoxyl signal at δ 3.77 (3H, s) was also observed in the ^{1}H NMR spectrum. Analyzing the 2D NMR spectra using HMQC and HMBC techniques enabled the assignment of ^{1}H and ^{13}C NMR signals. By comparing the NMR data of **7** with those of the known compound, garcinisidone-A,[4] the possible structure of **7** was established suggesting the same core structure for both compounds.

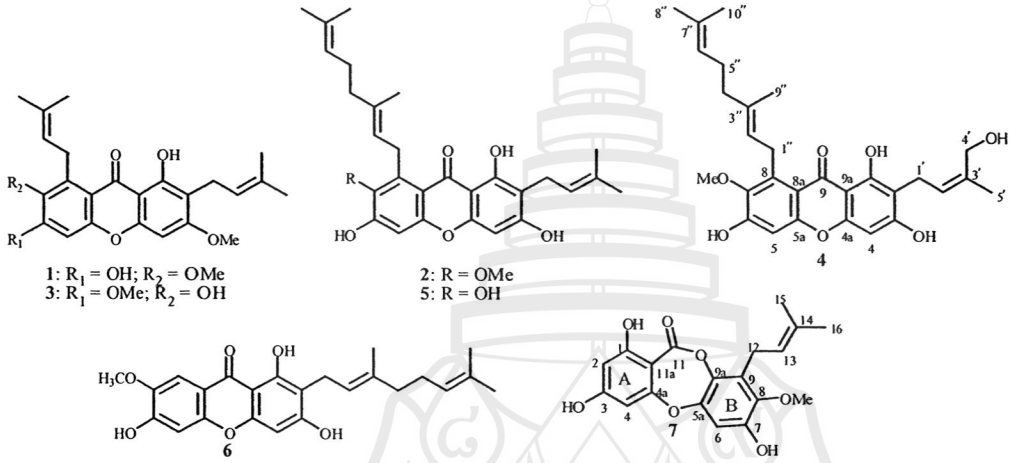

Figure 1. Structures **1-7**

Table 1. NMR spectral data (400 MHz, $CDCl_3$) of **7** in $CDCl_3$

No.	δ_H (mult., J in Hz)	δ_C	HMBC (^{1}H→^{13}C)
1	10.98 (s)	165.5 (s)	-
2	6.27 (br s)	100.6 (d)*	1, 3, 4, 11a
3	-	163.7 (s)	-
4	6.27 (br s)	100.5 (d)*	2, 3, 4a, 11a
4a	-	161.8 (s)	-
5a	-	146.5 (s)	-
6	6.70 (s)	105.5 (d)	5a, 8, 9a
7	-	147.0 (s)	-
8	-	142.7 (s)	-
9	-	128.3 (s)	-
9a	-	136.0 (s)	-
11	-	168.0 (s)	-
11a	-	98.8 (s)	-
12	3.47 (d, 6.8)	24.1 (t)	8, 9, 9a, 13, 14
13	5.17 (br s)	121.2 (d)	9, 15, 16
14	-	133.2 (s)	
15	1.80 (s)	18.0 (q)	13, 14, 16
16	1.67 (s)	25.7 (q)	13, 14, 15
OMe	3.77 (s)	61.8 (q)	8

* Interchangeable

The HMBC correlations of the methoxyl protons at δ 3.77 with the oxygenated carbon at δ 142.7 (C-8) and those of the methylene protons of a prenyl unit at δ 3.47 (H_2-12) with the carbons at δ 142.7 (C-8), 136.0 (C-9a), and 128.3 (C-9) established the attachment of the methoxyl group and the prenyl side chain at C-8 and C-9, respectively. The aromatic proton at δ 6.70 (H-6) showed HMBC connectivity to three aromatic carbons at δ 146.5 (C-5a), 142.7 (C-8) and 136.0 (C-9a), confirming the location of substituents on the B ring. Furthermore, the correlation of aromatic protons at δ 6.27 (H-2, H-4) with the aromatic carbons at δ 165.5 (C-1), 163.7 (C-3), 161.8 (C-4a), 100.6 (C-2), 100.5 (C-4) and 98.8 (C-11a) indicated the orientation of substituents on the A ring. The quaternary carbon signals of δ 165.5 (C-1), 163.7 (C-3), 147.0 (C-7) and its molecular formula $C_{19}H_{18}O_7$ indicated the presence of three hydroxy groups at C-1, C-3 and C-7, respectively. Thus, compound 7 was determined as cowadepsidone which reported for the first time as a metabolite of *G. cowa*. The remaining compounds were identified as β-mangostin (**1**),[9] cowanin (**2**),[10] 3,6-di-*O*-methyl-γ-mangostin (**3**),[11] cowanol (**4**),[10] norcowanin (**5**),[12] and cowaxanthone (**6**)[10] by the analysis of 1D and 2D NMR spectra and by comparison with their reported physical and spectroscopic data.

Table 2. Cytotoxicity of compounds **2-7**

Compounds	Cytotoxicity (μg/mL)		
	NCI-H187	KB	MFC-7
2	7.03	7.36	21.38
3	8.58	6.64	10.59
4	37.26	32.34	34.62
5	5.92	6.43	18.85
6	3.87	15.43	15.45
7	31.47	inactive	36.03
ellipticin	1.06	-	-
doxorubicin	-	9.61	9.17

As summarized in Table 2, compounds **2-7** were evaluated for their cytotoxicity against KB (oral human epidermal carcinoma), MCF-7 (breast cancer) and NCI-H187 (human, small cell lung cancer) cancer cell lines and all of them exhibited cytotoxic effect against all three cancer cell lines, except a new depsidone **7** was found to be inactive with KB cancer cell line (Table 2). Xanthones **2**, **3** and **5** exhibited strong cytotoxicity against KB cancer cell line with the IC_{50} value ranging from 6.43-7.36 μg/mL, which were stronger than doxorubicin, a standard drug, (IC_{50} 9.61 μg/mL) while **4** and **6** were found to be weakly activity. Xanthones **2**, **3**, **5** and **6** also showed strong inhibitory effect against NCI-H187 cancer cell line with the range of IC_{50} 3.87-8.58 μg/mL and xanthone **6** had the highest cytotoxicity (IC_{50} 3.87 μg/mL) whereas **4** and **7** exhibited weak activity. In case of cytotoxicity against MCF-7 cancer cell line, xanthone

3 was the best cytotoxicity with the IC_{50} value of 10.59 μg/mL. All the rest of compounds were found to be moderately to weakly active with the IC_{50} values ranging from 15.45-36.03 μg/mL.

It is interesting to note that the structural difference between xanthones **2** and **4** is only at C-4′. Xanthone **4** possesses a methylenehydroxyl moiety while **2** has a methyl group which plays an important role in the cytotoxicity against all three human cancer cell lines. In case of xanthones **2** and **5**, (**2** possesses a methoxyl group at C-7, while **5** contains a hydroxyl group), both of methoxyl and hydroxyl groups seemed to be no effective in the cytotoxicity.

EXPERIMENTAL

GENERAL

UV spectra were recorded with a Perkin-Elmer UV-Vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker spectrometer. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The ESITOFMS was obtained from a Micromass LTC mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5-40 μm) and silica gel 100 (Merck, 63-200 μm), respectively. Precoated plates of silica gel 60 F254 were used for analytical purposes.

PLANT MATERIAL

The twigs of *G. cowa* were collected from Nong Khai Province in March 2010. The plant specimen (MFU-NPR 0014) has been deposited at Natural Products Chemistry Laboratory, Mae Fah Luang University, Chiang Rai, Thailand.

EXTRACTION AND ISOLATION

The air-dried twigs of *G. cowa* (3.34 kg) were successively extracted with *n*-hexane and acetone over a period of 3 days each at room temperature. The *n*-hexane extract (21.36 g) was subjected to QCC over silica gel eluted with a gradient of *n*-hexane- EtOAc (100% *n*-hexane to 100% EtOAc) to provide five fractions (A-E). Fraction B (145.9 mg) was further purified by CC with 10% acetone- *n*-hexane to give compound **3** (6.8 mg) whereas compounds **1** (17.1 mg) and **2** (6.1 mg) were obtained from fraction D (124.2 mg) by repeated CC with 15% acetone-*n*-hexane. Fraction E (1.63 g) was subjected to repeated CC with 20% acetone- *n*-hexane to afford compounds **4** (165.2 mg), **5** (40.1 mg), **6** (30.2 mg) and **7** (10.1 mg).

Cowadepsidone (7): Red gum; UV ($CHCl_3$) λ_{max} (log ε): 204 (4.63), 273 (4.10), 313 (3.51), 433 (2.77); IR (neat) ν_{max} cm^{-1}: 3363 (OH), 1658 (C=O); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; ESITOFMS (*m/z*): $[M+H]^+$ *m/z* 359.1133 (calcd for $C_{19}H_{19}O_7$, 359.1131).

CYTOTOXIC ASSAY

The procedures for cytotoxic assay were performed by resazurin microplate assay (REMA) which was a modified method of fluorescent dye for the mammalian cell cytotoxicity according to Brien *et al.*[13] In this study, three cancer cell lines, KB (oral cavity cancer), MCF7 (breast cancer) and NCI-H187 (small cell lung cancer) were used. Ellipticin and doxorubicin were the reference substances in this study and the IC_{50} values are summarized in Table 2.

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ABSTRACT

Garcinia genus, belonging to Clusiaceae family, usually produced phenolic compounds such as xanthones, phologucinols. *G. cowa* is one of the species in this genus. The six known phenolic xanthones namely cowanin, cowanol, α-mangostin, 1,7-dihydroxyxanthone, 1,3,7-trihydroxy-2-prenylxanthone and 1,3,6,7-tetrahydroxyxanthone and a new depsidone namely cowadepsidone were isolated from *G. cowa*, collected from Nongkhai province Northeast part of Thailand. Their structures were characterized by spectroscopic techniques. In addition, antibacterial activity of the isolates was also evaluated. Cowanin, cowanol and 1,3,6,7-tetrahydroxyxanthone were found to be strong activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) SK1 with MIC values of 4, 2 and 4 μg/mL, respectively.

Keywords: *Garcinia cowa*, xanthone, acylphloglucinol, depsidone, antibacterial activity.

บทคัดย่อ

พืชสกุลมังคุดผลิตสารส่วนใหญ่เป็นสารที่มีหมู่ฟีนอลลิก เช่น แซนโทน ฟลูโรกลูซินอล ส้มโมง เป็นพืชอีกหนึ่งชนิดที่จัดอยู่ในสกุลเดียวกับมังคุด จากการศึกษากิ่งส้มโมงที่เก็บจากจังหวัดหนองคาย ภาคตะวันออกเฉียงเหนือของประเทศไทย พบว่าพืชชนิดนี้ผลิตสารส่วนใหญ่เป็นสารประกอบประเภทแซนโทน ไม่พบสารประกอบในกลุ่มของฟลูโรกลูซินอลอยู่เลย แต่กลับพบสารใหม่ที่จัดอยู่ในสารประเภทเดพซิโดน 1 ชนิดชื่อว่าโคว่าเดพซิโดน นอกจากนี้สารที่แยกได้ยังนำไปทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย ทั้งชนิดแกรม-เนกาทีฟ กับ แกรม-โพซิทีฟ พบว่าสารประกอบประเภทแซนโทนสามชนิดคือโควานิน โควานอล และ 1,3,6,7 เตตระไฮดรอกซีแซนโทนออกฟทธิ์ต้านเชื้อแบคทีเรียชนิด Methicillin-resistant *Staphylococcus aureus* (MRSA) SK1 ได้ดีมาก

TABLE OF CONTENT

LIST OF TABLE

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

cm^{-1}	wave numbers
cm	centimeter
g	gram
mg	milligram
µg/mL	microgram per milliliter
IC_{50}	half maximal inhibitory concentration
EC_{50}	half maximal effective concentration
MIC	minimum inhibitory concentratio
FT-IR	Fourier transform infrared spectroscopy
^{1}H NMR	proton Nuclear Magnetic Resonance
^{13}C NMR	carbon Nuclear Magnetic Resonance
QCC	Quick Column Chromatography
CC	Column Chromatography
s	singlet
d	doublet
t	triplet
dd	doublet of doublet
S.A.	*Staphylococcus aureus* TISTR 1466
MRSA	Methicillin-resistant *S. aureus*
E.C	*Escherichia coli* TISTR 780
S.T.	*Salmonellae typhimurium* TISTR 292

CHAPTER 1

INTRODUCTION

The plants of *Garcinia* genus, a small to medium sized evergreen tree wildly spread in lowland, undulating areas and peat swamp forests in asia, southern africa, and polynesia, bolong to the Clusiaceae family. *Garcinia cowa*, commonly known as 'Cha-muang in Thai or Som-Muang in Northeast part of Thailand, is one of more than 300 species in the genus *Garcinia*.

The leaves simple, opposite, 6-15 by 2.5-6 cm, oblong, thick and glossy, usually less than 3 times as long as wide with blunt or slightly pointed tips; petiole c 1 cm. *Inflorescence* a cluster of single to few flowered, axillary, flower unisexual. *Male flower* without stigma, the stamens in single squarish mass. *Female flower* with shallowly 4-8 ridged stigma. Fruit*s* is globose, 2.5-6 cm, green when young, dull orange or yellow at maturity with 5-8 shallow grooves at least near the top. Tip sunken with small black persistent calyx, 4-8 segments. *Seed* large 3-angled.

Figure 1-1. *Garcinia cowa* pictures, Photo: Asst. Prof. Surat Laphookhieo.

This tree is common in most lowland forest types. It is found in low sand dune forest behind the beach, in tropical evergreen forest, or in dry deciduous forest throughout the country. Young leaves are cooked in famous dish "tom mu chamuang"(pork curry) of the provinces in the southeast.

The secondary metabolites of this genus have been reported to possess a wild range of biological activity, such as anti-cancer agent anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, antiulcer and antioxidant.[1] Due to the promising of wildly broad spectrum of pharmacological properties, it renders the plant of *G. cowa* an attractive and challenging target for bioactive compounds. Interestingly, either different part of the plant or collection from different place gave quite unique phytochemical constituents. Many parts of *G. cowa* have been used as traditional folk medicine, for example, leaves and latex for antifever agent.

The aim of the present study is thus to isolate the secondary metabolites from *G. cowa*, analyse chemical structure and test biological activity. We report herein six known xanthones, cowanin **(1)**, cowanol **(2)**, α-mangostin **(16)** , 1,7-dihydroxyxanthone **(28)**, 1,3,6,7-tetrahydroxyxanthone **(30)**, 1,3,7-trihydroxy-2-prenylxanthone **(50)** and a new depsidone namely cowadepsidone **(51)** isolated from *G. cowa*. Anti-bacterial activity of compounds **1**,**2**,**16**,**30**,**50** and **51** was also evaluated.

CHAPTER 2
LITERATURE REVIEWS

Previous investigation of the *G. cowa* revealed that xanthones and acylphloroglucinol derivatives are one of the richest sources of natural chemotaxonomy having unique structural diversity. The distinctive chemical structure of xanthones is represented as two aromatic rings fused by a carbonyl carbon and oxygen linker as shown in Figure 2.

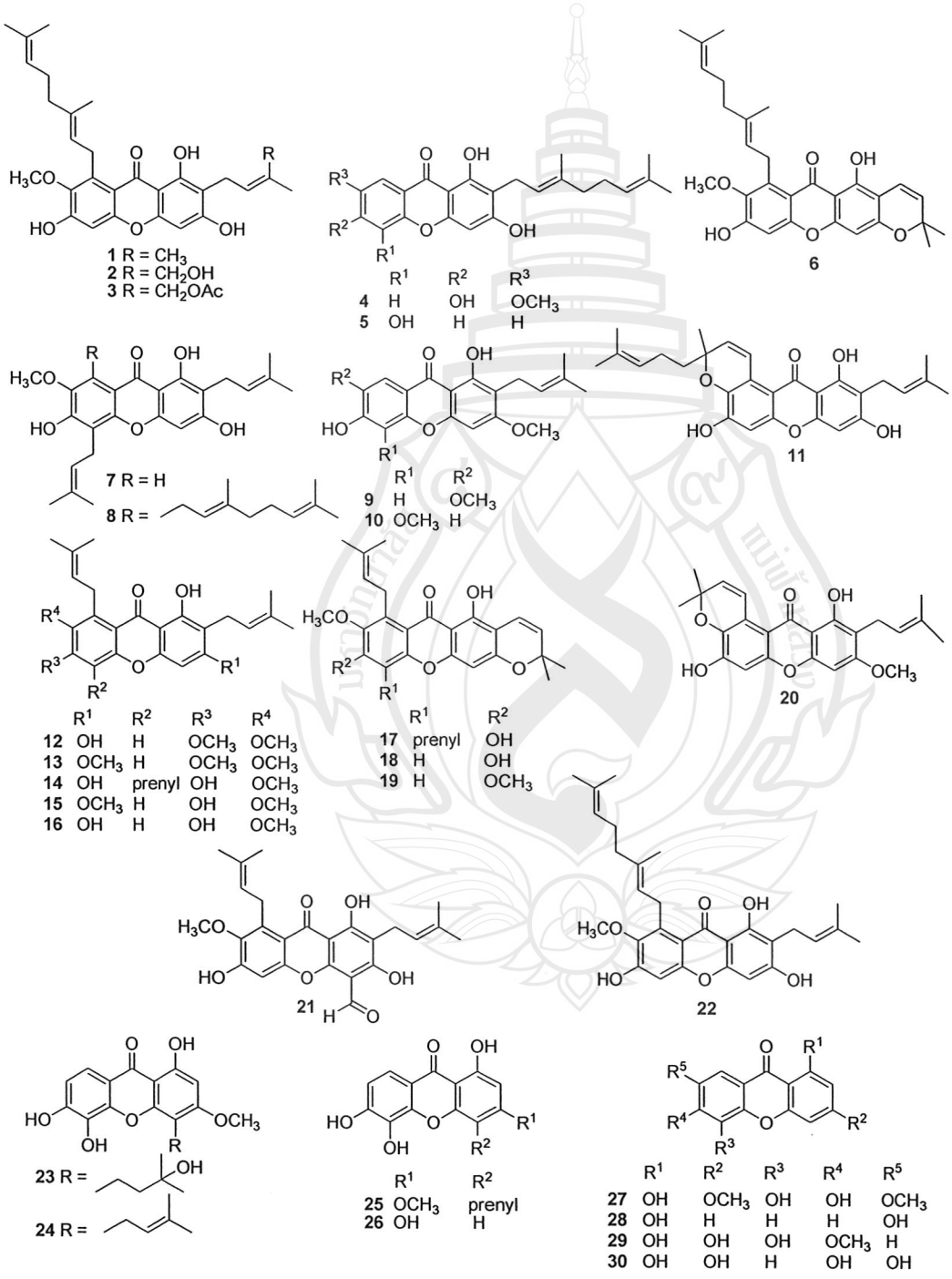


Figure 2-1. Secondary metabolite prenylated xanthones isolated from *G.cowa.*

The ring system may be substituted with a variety of prenyl, geranyl, phenilic, methoxy groups that give a large variety of possible structure. Generally, hydroxyl group of all isolated from *Garcinia species* is mainly located quite uniquely with respect to substitution at C-1 of xanthone core structure.

Table 2-1. Naturally occurring xanthones from the *G. cowa*.

Structural name	Plant part	Referenc
Cowanin **(1)**	Latex	2
Cowanol **(2)**	Latex	2
Cowagarcinone E **(3)**	Latex	2
Cowaxanthone **(4)**	Latex	2
Mangostinone **(5)**	Latex	2
Fucaxanthone A **(6)**	Latex	2
1,3,6-Trihydroxy-7-methoxy-2,5-bis-(3-methyl-2-butenyl)xanthone **(7)**	Latex	2
Cowagarcinone A **(8)**	Latex	2
Cowagarcinone B **(9)**	Latex	2
Cowagarcinone C **(10)**	Latex	2
Cowagarcinone D **(11)**	Latex	2
Cowaxanthone B **(12)**	Fresh fruits	3
Fuscaxanthone C **(13)**	Fresh fruits	3
7-O-Methylgarcinone E **(14)**	Fresh fruits	3
β-Mangostin **(15)**	Fresh fruits	3
α-Mangostin **(16)**	Fresh fruits	3
Cowaxanthone C **(17)**	Fresh fruits	3
Mangostanin **(18)**	Fresh fruits	3
6-*O*-methylmangostanin **(19)**	Fresh fruits	3
Cowaxanthone D **(20)**	Fresh fruits	3
Cowaxanthone E **(21)**	Fresh fruits	3
Cowanin **(22)**	Fresh fruits	3
1,5,6-Trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone **(23)**	stems	4

Table 2-1. (Continued)

Structural name	Plant part	Referenc
Dulxanthone A **(24)**	stems	4
1,5-Dihydroxy-3-methoxy-6',6'-dimethyl-2*H*-pyrano (2',3':6,7)-4-(3-methylbut-2-enyl)xanthone **(25)**	stems	4
1,3,5-Trihydroxy-6',6'-dimethyl-2*H*-pyrano-(2',3':6,7)xanthone **(26)**	stems	4
1,5,6-Trihydroxy-3,7-dimethoxyxanthone **(27)**	stems	4
1,7-Dihydroxyxanthone **(28)**	stems	4
1,3,5-Trihydroxy-6-methoxyxanthone **(29)**	stems	4
1,3,6,7-tetrahydroxyxanthone **(30)**	stems	4

Acylphloroglucinol, phloroglucinol moiety connected with benzoyl synthon, is general precursor of biosynthetic pathway of xanthone as shown in Table 2 and Figure 3. The polyprenylated unit is usually substituted as core moiety with one or more prenyl or geranyl side chains. Garcinol,[6] one of the representative acylphloroglucinol derivatives, has been extensively studied for induced appotosis and inhibit cell survival and proliferation pathways such as NAPK and PI3K/Akt.[7]

Table 2-2. Naturally occurring acylphloroglucinols from the *G. cowa*.

Structural name	Plant part	Reference
garcicowins A **(31)**	Twigs	5
garcicowins B **(32)**	Twigs	5
garcicowins D **(33)**	Twigs	5
30-epicambogin **(34)**	Twigs	5
Cambogin **(35)**	Twigs	5
garcicowins C **(36)**	Twigs	5
Oblongifolins A, B **(37)**	Twigs	5
Oblongifolins C **(38)**	Twigs	5
Guttiferone B **(39)**	Twigs	5
Oblongifolin D **(40)**	Twigs	5
guttiferone K **(41)**	Twigs	5

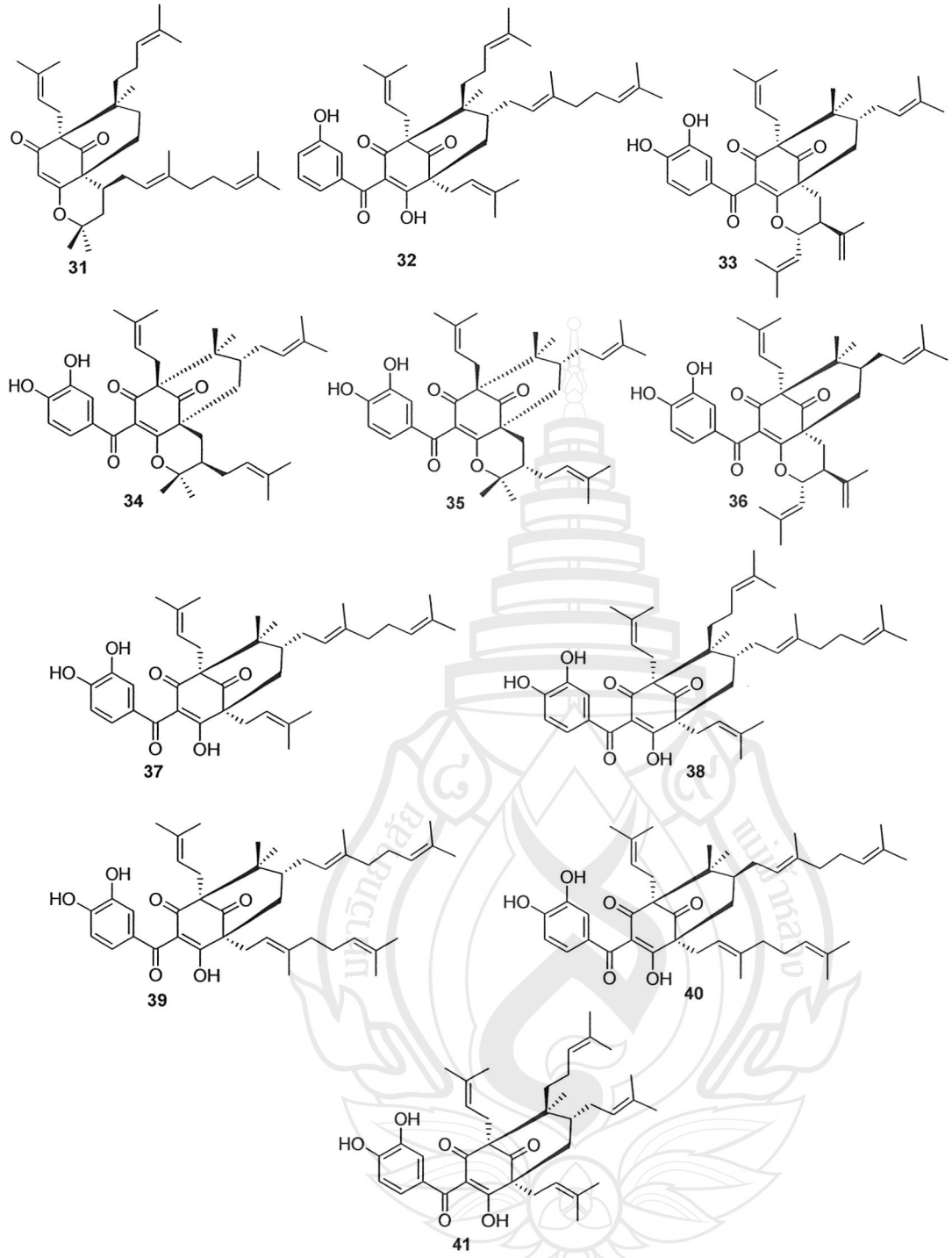
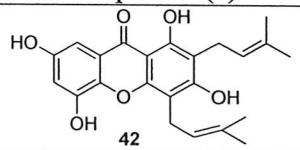
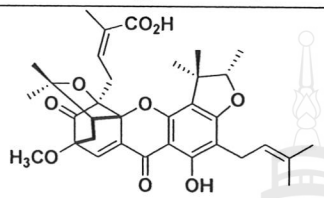
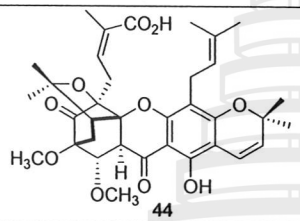
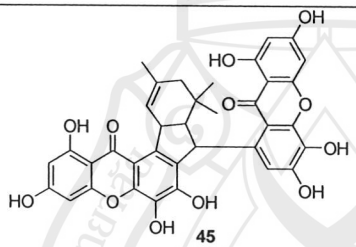
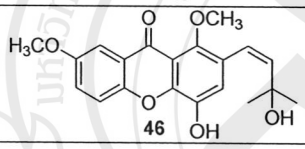
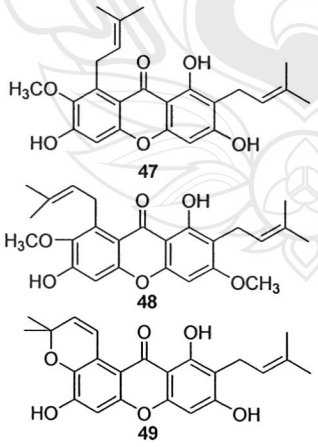


Figure 2-2. Secondary metabolite acylphloroglucinol derivatives isolated from *G.cowa.*

A number of xanthones and their derivatives have been continuously isolated from natural sources of *Garcinia* plants and some of them have been investigated for a variety of biological as shown in Table 3. It was found that cage xanthone **43** showed to be strong anti-bacterial with MIC values of 3.38 μg/mL.

Table 2-3. Representative bioactive xanthones isolated from Garcinia genus.

Species	source	Active cytotoxic component(s)	activity	Ref
G.staudtii	twigs	 42	antibacterial activity (MIC 16μg/mL)	7
G. scortechinii	Fruits	 43	antibacterial activity (MIC 3.38μg/mL)	8
G. hanburyi	Fruits	 44	antibacterial activity (MIC 25 μg/mL)	9
G. virgata	Stem barks	 45	antioxidant activity (EC_{50} 11.5µg/ 100 mL)	10
G. afzelii	stem barks	 46	antioxidant activity (IC_{50} 14 µg/ 100 mL)	11
G. mangostana	Fruits	 47 48 49	against mycobacterim tuberculosis	12

CHAPTER 3

MATERIAL AND METHODS

Plant Material

The twigs of *G. cowa* were collected from Nongkhai province in Northeast part of Thailand in March 2010, and identified by Mr. James Maxwell, Chiang Mai University Herbarium. A voucher specimen (MFU-NPR 0012) was deposited at Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100 Thailand.

General experimental

Melting points were determined by Perkin-Elmer FTS FT-IR spectrophotometer and are uncorrected. The IR spectra were recorded with Perkin-Elmer FTS FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded by 400 MHz Bruker spectrometer. Tetramethylsilane (TMS) are used as internal reference. The MicroTOF, Bruker Daltonics mass spectrometer was used to correct ESI-TOF-MS data. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5-40 μm) and silica gel 100 (Merck, 63-200 μm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Extraction and Isolation

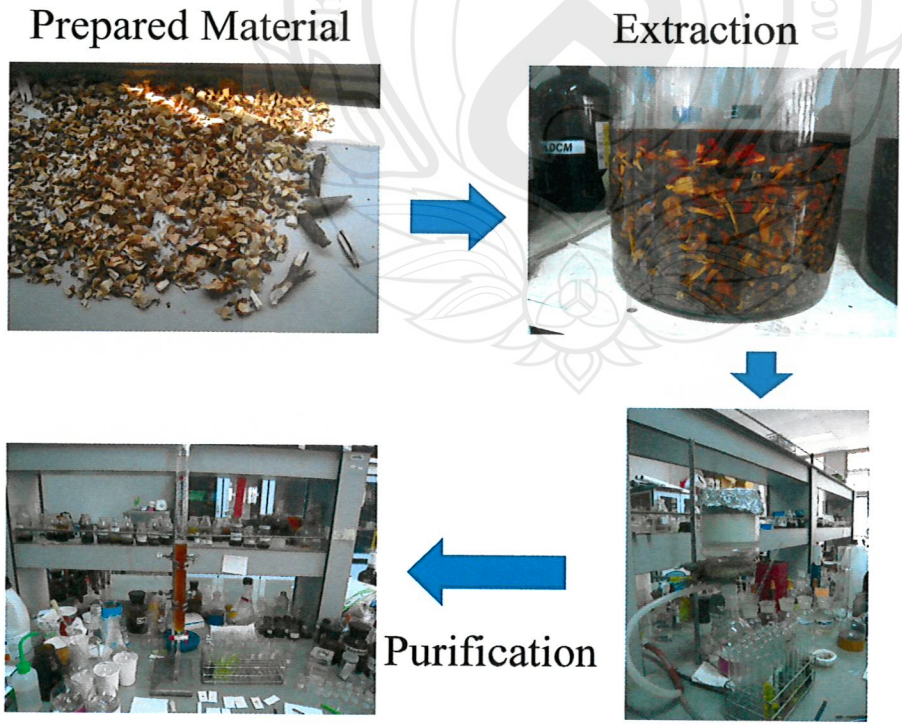
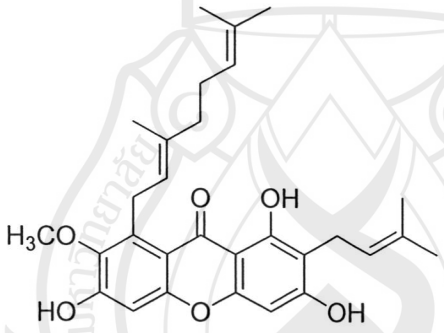


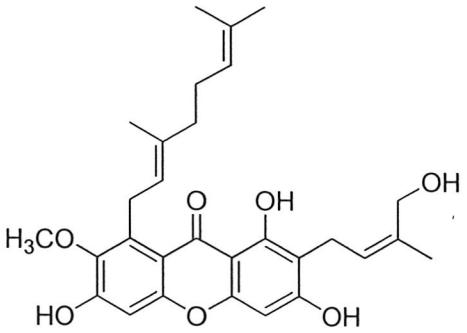
Figure 3-1. Purification flow-chart of secondary metabolites from *G. cowa*.

The twigs of *G. cowa* (3.34 kg) were extracted with acetone over the period of 3 days at room temperature. Removal of solvent under reduced pressure provided acetone extract (140 g) which was chromatographed by QCC, and eluted with a gradient of hexanes-acetone (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) to afford 18 fractions (GCTA-A (621 mg), GCTA-B (40 mg), GCTA-C (19 mg), GCTA-D (14 mg), GCTA-E (315 mg), GCTA-F (883 mg), GCTA-G (1.49 g), GCTA-H (3.13 mg), GCTA-I (1.28 mg), GCTA-J (927 mg), GCTA-K (212 mg), GCTA-L (654 mg), GCTA-M (3.46 g), GCTA-N (9.69 mg), GCTA-O (7.14 g), GCTA-P (2.18 g), GCTA-Q (1.98 g) and GCTA-R (3.14 g). Fraction GCTA-F (1.6 g) was purified by CC with 25% EtOAc-hexanes to afford compounds **1** (22 mg) and **28** (23.5 mg). Compounds **1** (5.1 mg), **2** (136.9 mg), **16** (9.5 mg), **50** (9.2 mg), **51** (11.6 mg) were derived from fraction GCTA-H by repeated CC using 30% EtOAc-hexanes. Fraction GCTA-K was filtered and washed with cold methanol to give **30** (159 mg)



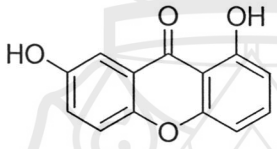
Cowanin **(1)**

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.54 (S, 3H), 1.59 (s, 3H), 1.64 (s, 3H), 1.99-2.06 (m, 4H), 3.42 (d, J = 7.2 Hz, 2H), 3,79 (s, 3H, OCH_3), 4.08 (d, J = 6.0 Hz, 2H), 5.01 (t, J = Hz, 1H), 5.02-5.30 (m, 4H), 6.27 (s, 1H), 6.79 (s, 1H), 13.76 (s, 1H). ^{13}C NMR ($CDCl_3$, 100MHz): δ 16.5, 17.7, 17.9, 21.5, 25.6, 25.8, 26.5, 26.6, 29.7, 31.0, 39.7, 62.0, 93.2, 101.6, 103.6, 108.8, 112.2, 121.7, 123.3, 1124.3, 131.3, 135.0, 135.5, 137.2, 142.7, 154.6, 155.0, 155.7, 160.6, 161.6, 182.0.



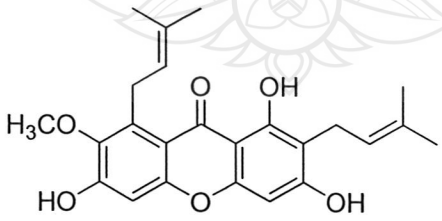
Cowanol **(2)**

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.5 (S, 3H), 1.60 (s, 3H), 1.68 (s, 3H),1.75 (s, 3H), 1.99-2.10 (m, 4H), 3.45 (d, J = 7.6 Hz, 2H), 3,80 (s, 3H, OCH_3), 4.13 (d, J = 6.4 Hz, 2H),4.33 (s, 2H), 5.05 (t, J = Hz, 1H), 5.29 (t, J = 6.4 Hz, 1H), 5.36 (t, J = 7.6 Hz, 1H), 6.36 (s, 1H), 6.81 (s, 1H), 13.90 (s, 1H).



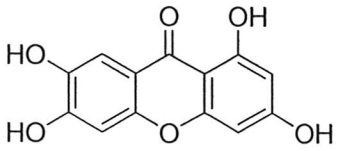
1,7-dihydroxyxanthone **(28)**.

^{1}H NMR ($CDCl_3$+CD_3OD, 400MHz): δ 6.73 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.31 (dd, J = 9.2, 2.4 Hz, 1H), 7.40 (d, J = 9.2 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), 7.60 (t, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$+CD_3OD, 100MHz): δ 107.8, 109.1, 109.2, 110.3, 119.9, 121.8, 126.0, 137.4, 151.1, 154.9, 157.4, 162.3, 183.2.



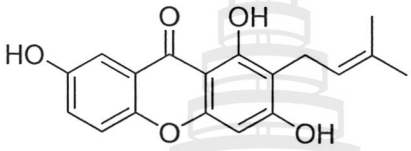
α-mangostin **(16)**

^{1}H NMR ($CDCl_3$, 400MHz): δ 1.71 (s, 3H), 1.79 (s, 3H), 1.86 (s, 3H), 1.87 (s, 3H), 3.47 (d, J = 6.8 Hz, 2H), 3.82 (s, 3H, OCH3), 4.11 (d, J = 6.0 Hz, 2H), 5.29 (m, 2H), 6.31 (s, 1H), 6.84 (s, 1H), 13.8 (s, 1H).



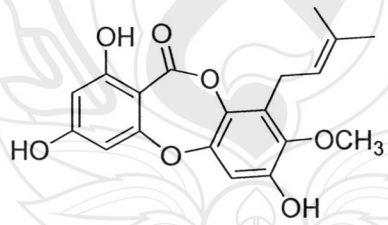
1,3,6,7-tetrahydroxy xanthone **(30)**

^{1}H NMR (DMSO-d6, 400MHz): δ 6.15 (d, *J* = 2.0 Hz, 1H), 6.32 (d, *J* = 2.0 Hz, 1H), 6.86 (s, 1H), 7.37 (s, 1H), 10.46 (br s, 3H), 13.1 (s, 1H). ^{13}C NMR (DMSO-d6, 100MHz): δ 94.1, 98.1, 102.0, 103.1, 108.5, 112.2, 144.2, 151.4, 154.5, 157.8, 163.1, 165.1, 179.3.



1,3,7-trihydroxy-2-prenylxanthone **(50)**

^{1}H NMR (Acetone-*d6*, 400MHz): δ 1.75 (s, 3H), 1.88 (s, 3H), 3.46 (d, *J* = 8.0 Hz, 2H), 5.38 (t, *J* = 8.0 Hz, 1H), 6.59 (s, 1H), 7.43 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.675 (d, *J* = 4.0 Hz, 1H), 13.36 (s, 1H).



Cowadepsidone **(51)**: Red gum; UV ($CHCl_3$) λ_{max} (log ε): 204 (4.63), 273 (4.10), 313 (3.51), 433 (2.77); IR (neat) ν_{max} cm^{-1}: 3363 (OH), 1658 (C=O); ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 4-1; ESITOFMS (*m/z*): $[M+H]^+$ *m/z* 359.1133 (calcd for $C_{19}H_{19}O_7$, 359.1131).

CHAPTER 4

RESULTS AND DISCUSSION

The acetone extract of the twigs of *G. cowa* was purified by column chromatographic technique to give six oxygenated xanthones including cowanin **(1)**,[13] cowanol **(2)**,[13] *α*-mangostin **(16)**,[14] 1,7-dihydroxyxanthone **(28)**,[15] 1,3,6,7-tetrahydroxyxanthone **(30)**,[16] 1,3,7-trihydroxy-2-prenylxanthone **(50)**[17] and one depsidone namely cowadepsidone **(51)**.[18] All compounds were elucidated using spectroscopic methods and compared with those reported in the literatures.

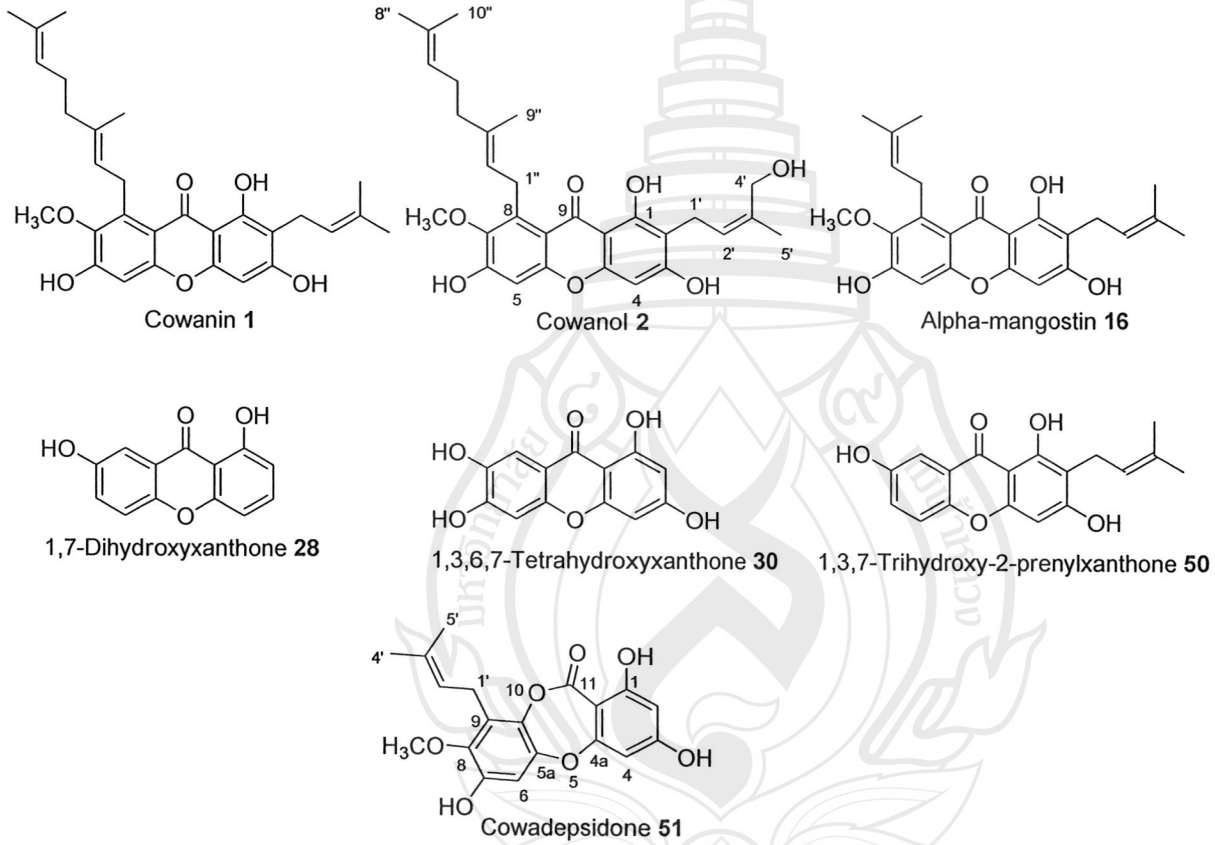


Figure 4-1. Secondary metabolite constituents isolated from *G. cowa*.

Cowanin **(1)** was a yellow solid with molecular formular $C_{29}H_{34}O_6$. The IR spectrum showed the hydroxyl and carbony functionalities at 3272 and 1607 cm^{-1}. The ^1H NMR spectrum displayed resonances of a singlet aromatic protons at δ 6.27 (1H, s) and δ 6.79 (1H, s) which were assigned as H-4 and H-5, respectively. A methoxy group was presented at δ 3.79 (3H, s). Moreover, prenylated group and geranyl moiety were located at C-2 and C-8 of xanthone ring. The methylene protons on prenyl group, H-1', appeared at δ 3.42 (d, J = 7.2

Hz, 2H), and methylene protons on geranyl moiety at H-1" were also indicated in the ^{1}H NMR spectrum at δ 4.08 (d, J = 6.0 Hz, 2H).

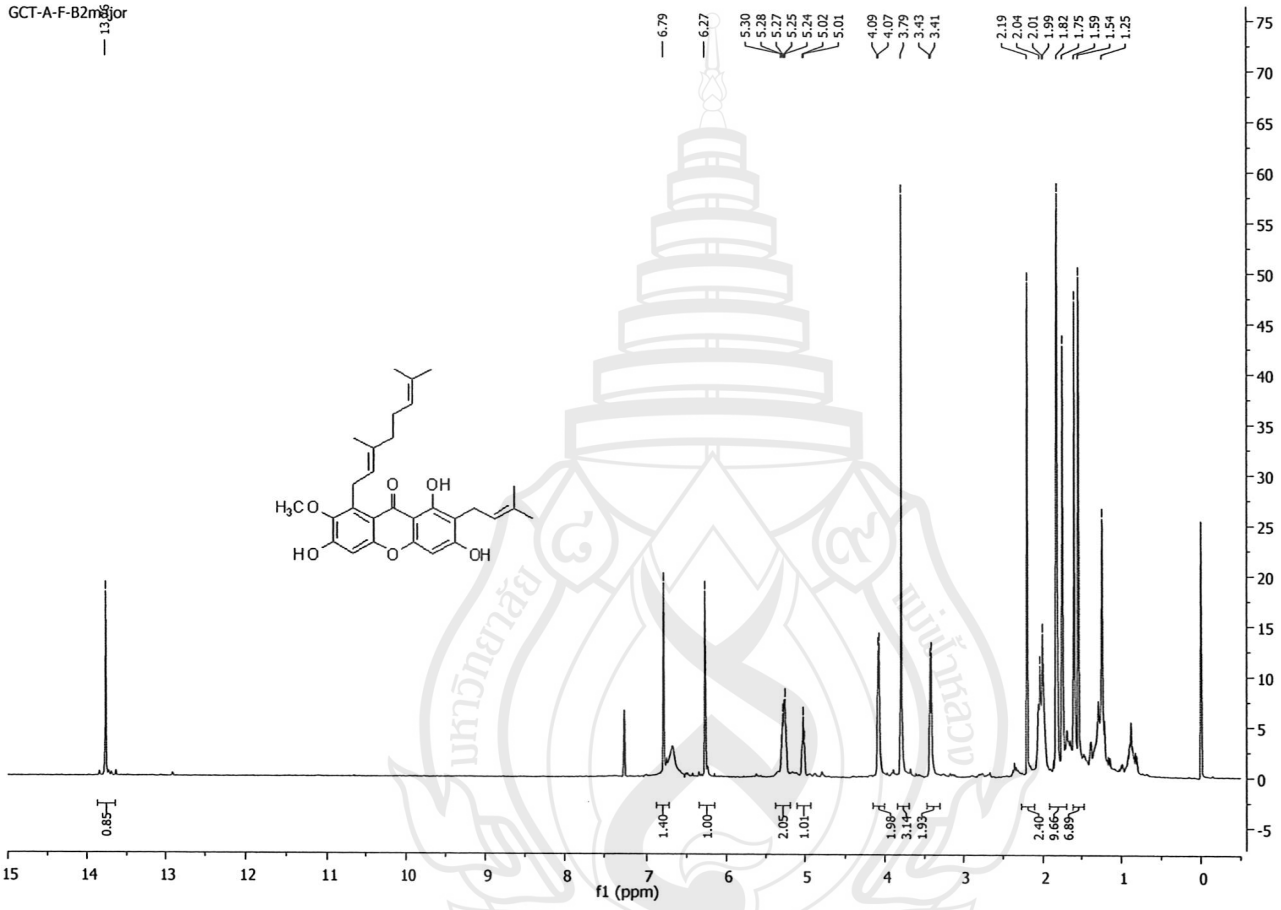


Figure 4-2. ^{1}H NMR of cowanin **(1)**.

This prenyl unit was located on C-2 of the xanthonoid skeleton due to the methylene protons H-1′(δ 3.42), showed cross peaks with the C-2 at δ 108.8 (1H, br s, H-7) in the, and Proton at H-4 δ 6.27 also correlated with C-2 based on analysis by ^{2}J and ^{3}J HMBC. The unit of the geranylnyl proton located at C-8 was also confirmed by ^{2}J and ^{3}J HMBC derived from the correlationships of H-1" [δ 4.08 (d, J = 6.0 Hz, 2H)] with C-8. The *O*-methoxy was placed on C-7 because of the *O*-methyl protons (δ 3.79) and H-5 (δ 6.79 (1H, s) showing the correlations with those C-7 (δ 142.7). Therefore, the structure of **1** was identified to be cowanin.

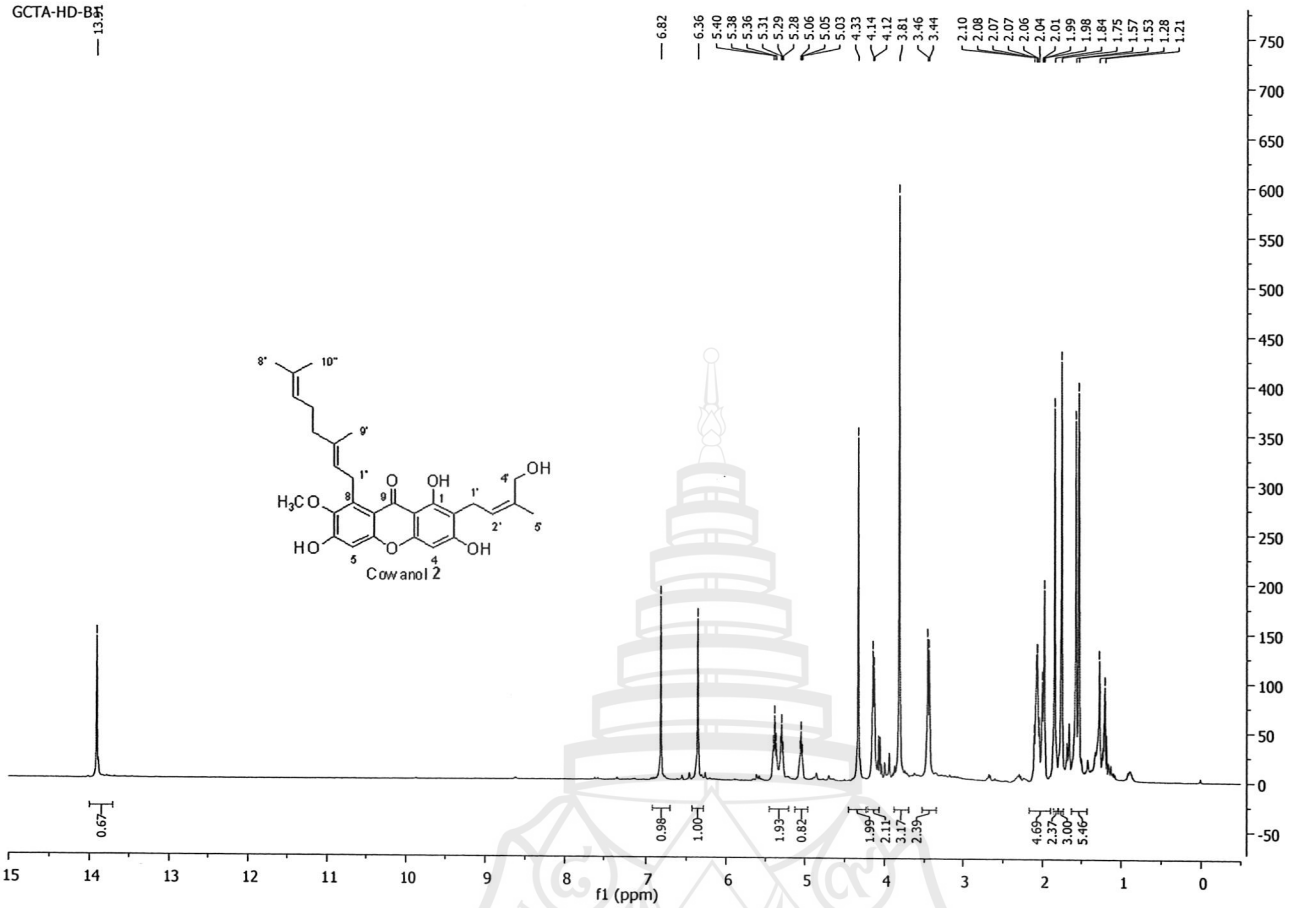


Figure 4-3. ^{1}H NMR of cowanol (**2**).

The framework of cowanol (**2**) was similar to those of cowanin (**1**) except for the fact that the methyl at C-5' of prenylated moiety was oxidized to primary alcohol. The ^{1}H-NMR showed almost identical with compound (**1**), but the five sets of singlet methyl protons from **1** was reduced to four sets resonance at δ 1.53, 1.57, 1.75 and 1.84. In addition, the methylene alcohol proton at H-5' was located at lower filed than those methyl set at δ 4.33 (s, 2H).

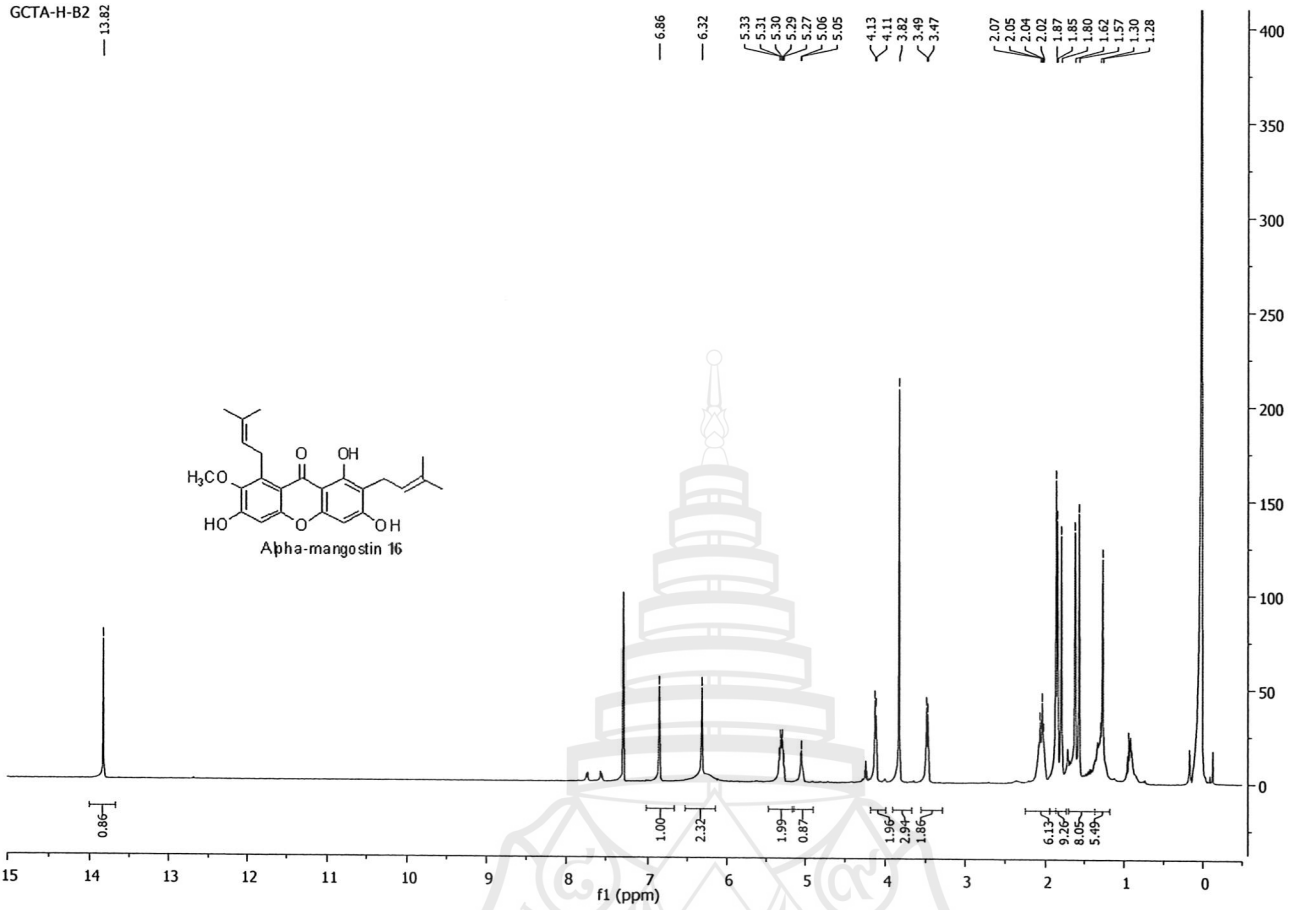


Figure 4-4. ^{1}H NMR of α-mangostin **(16)**.

The ^{1}H-NMR of α-mangostin is generally shown two sets of isoprenyl units located around δ 1.57-5.33, 1.57, 1.62, 1.80 and 1.87 (4xCH_3), 3.84 (d, J = Hz, H-1'), 4.12 (d, J = Hz, 1''), 5.30 (m, 2H, H-2', 2'') and a chelated hydroxyl group at 13.82 (s, 1H, C_1-OH). In addition, aromatic protons resonance at 6.32 (s, H-4) and 6.86 (s, H-5), and *O*-methoxy group represented at 3.82 (3H, OCH_3).

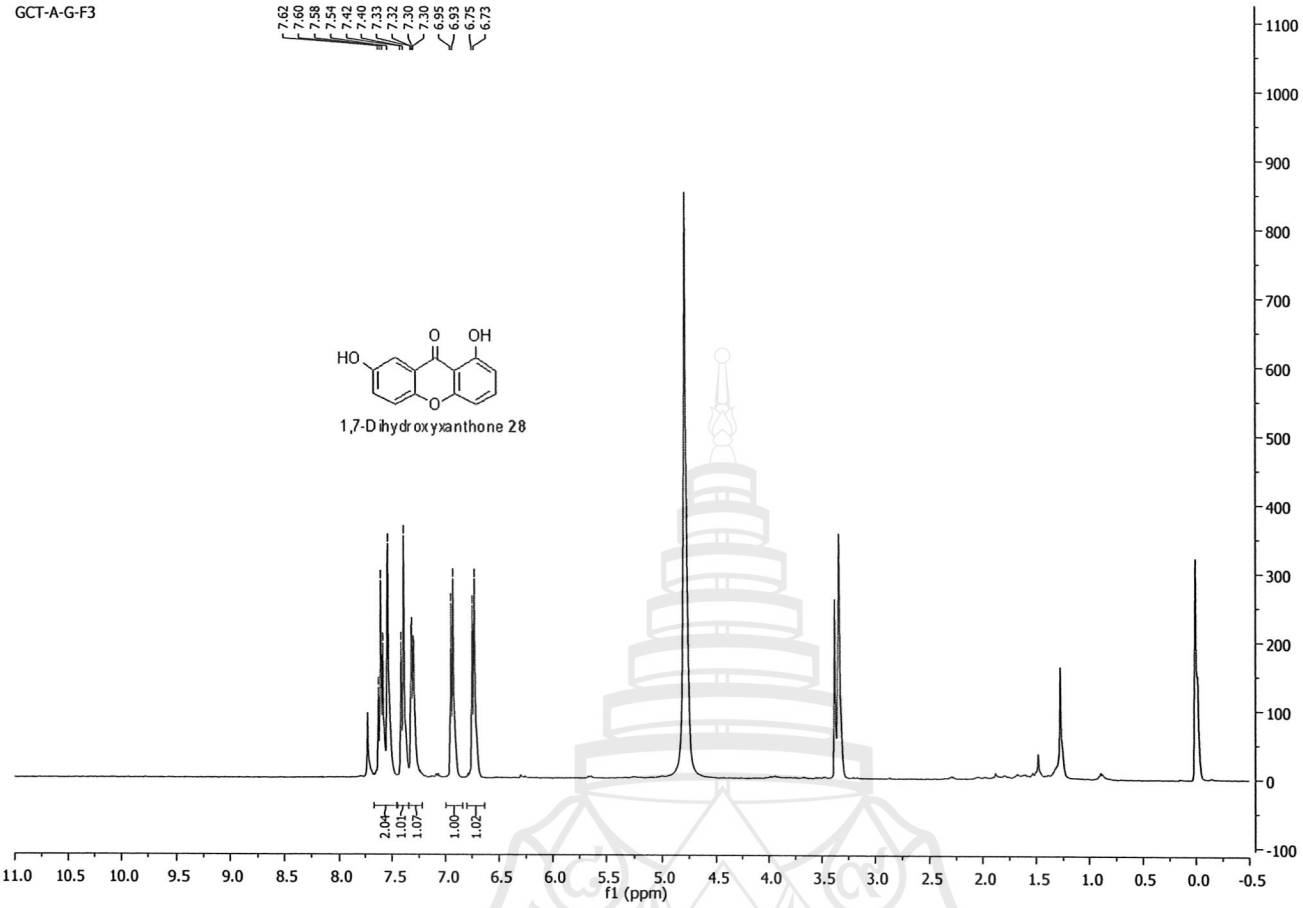


Figure 4 5. ^{1}H NMR of 1,7-dihydroxyxanthone **(28)**.

The ^{1}H NMR of 1,7-dihydroxyxanthone showed quite unique pattern on aromatic protons. They consisted of two sets of ^{1}H NMR data, ABC and AMX systems. The first set of ABC system was resonated at δ 6.73 (d, J = 8.4 Hz, 1H, H-4), 6. 93 (d, J = 8.4 Hz, 1H, H-2) and 7.60 (t, J = 8.4 Hz, 1H, H-3), and another set of AMX systems was displayed at 7.31 (dd, J = 9.2, 2.4 Hz, 1H), 7.40 (d, J = 9.2 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), assigning as H-6, H-5 and H-8, repectively.

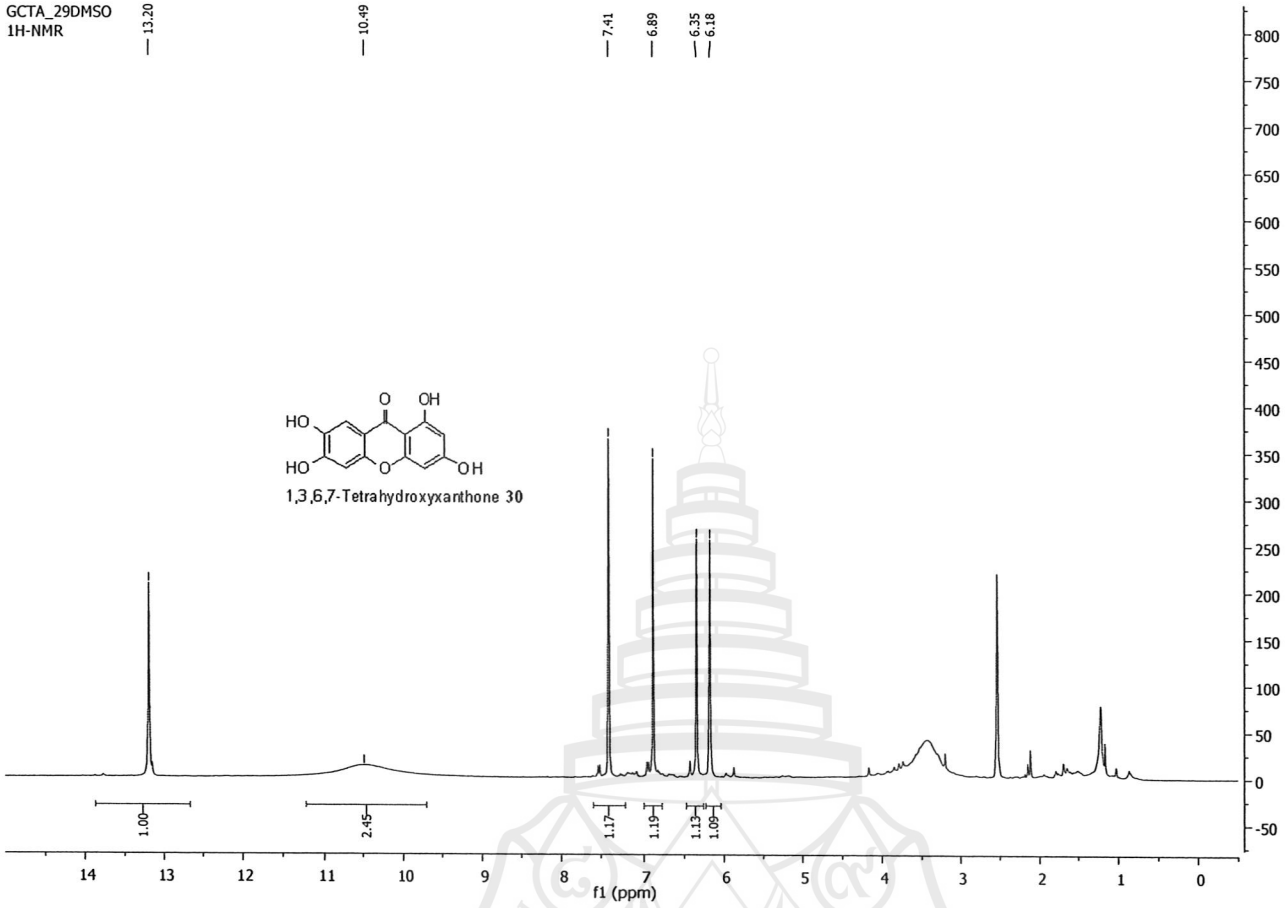


Figure 4-6. ^{1}H NMR of 1,3,6,7-tetrahydroxyxanthone **(30)**.

The distinctive ^{1}H NMR of 1,3,6,7-tetrahydroxyxanthone **30** can be distinguished by two sets of aromatic protons as followed. One was *para*-^{1}H-NMR replaced at δ 6.89 (1H, H-5) and δ 7.41 (1H, H-8), and another one was located at δ 6.18 (d, J = 2.0 Hz, H-4) and δ 6.35 (d, J = 2.0 Hz, H-2). Moreover, a chelated hydroxyl group was shown at lowest field, δ 13.20 (s, 1H, C_1-OH)

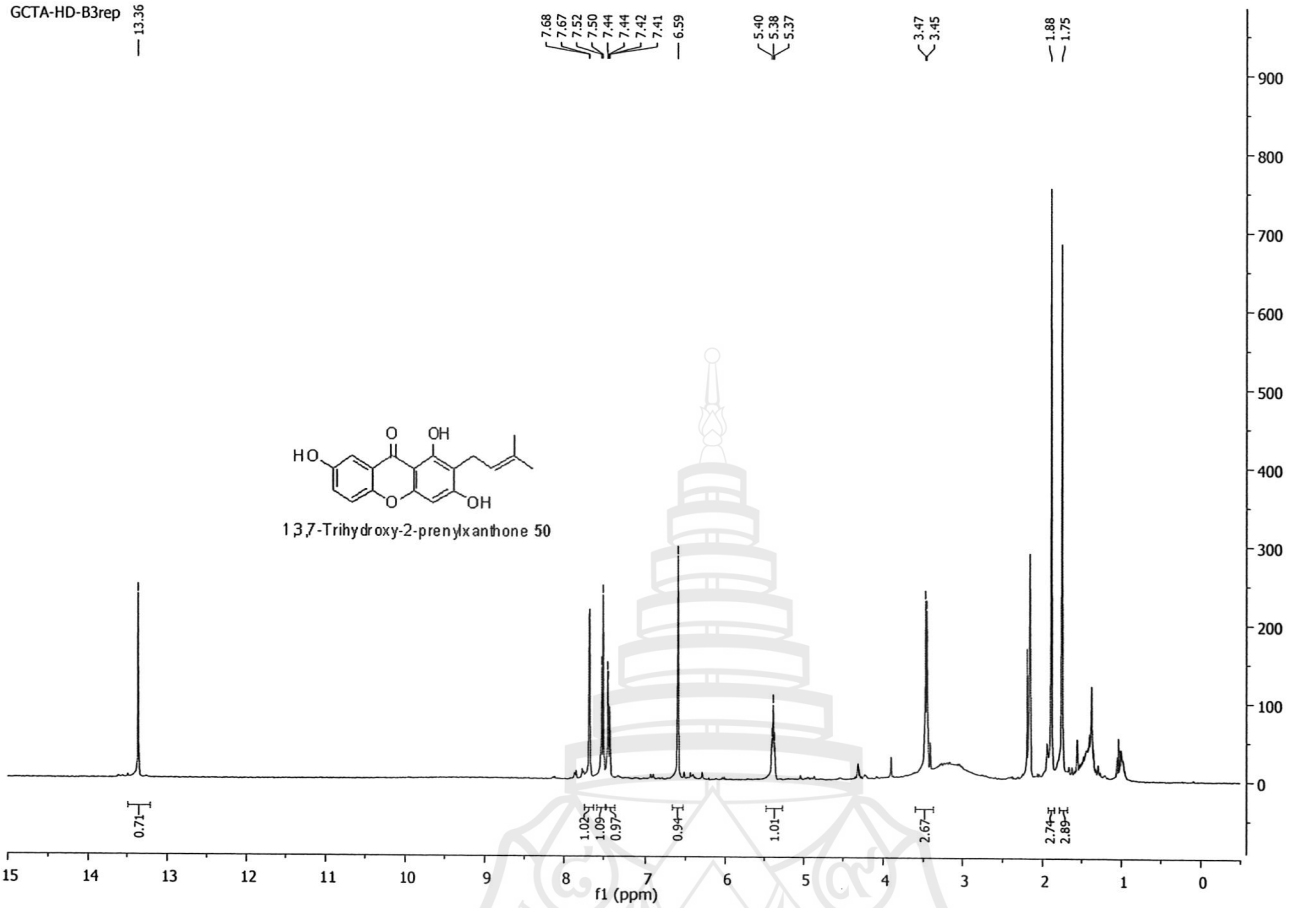


Figure 4-7. ^{1}H NMR of 1,3,7-trihydroxy-2-prenylxanthone **(50)**.

The analysis of compound **(50)** on the basis of 1H NMR data unveiled that this mololecule is one of the xanthone derivatives. The chelated hydrogen bonding at C_1-OH at δ 13.36 , and isoprenyl pattern [δ 1.75 (s, 3H, CH_3), 1.88 (s, 3H, CH_3), 3.46 (d, J = 8.0 Hz, 2H, H-1'), 5.38 (t, J = 8.0 Hz, 1H, H-2')] and the AMX system on aromatic ring [6.59 (s, 1H, H-4), 7.43 (dd, J = 8.0, 4.0 Hz, 1H, H-6), 7.51 (d, J = 8.0 Hz, 1H, H-5), 7.675 (d, J = 4.0 Hz, 1H, H-8), 13.36 (s, 1H)] are the characteristic data of xanthone.

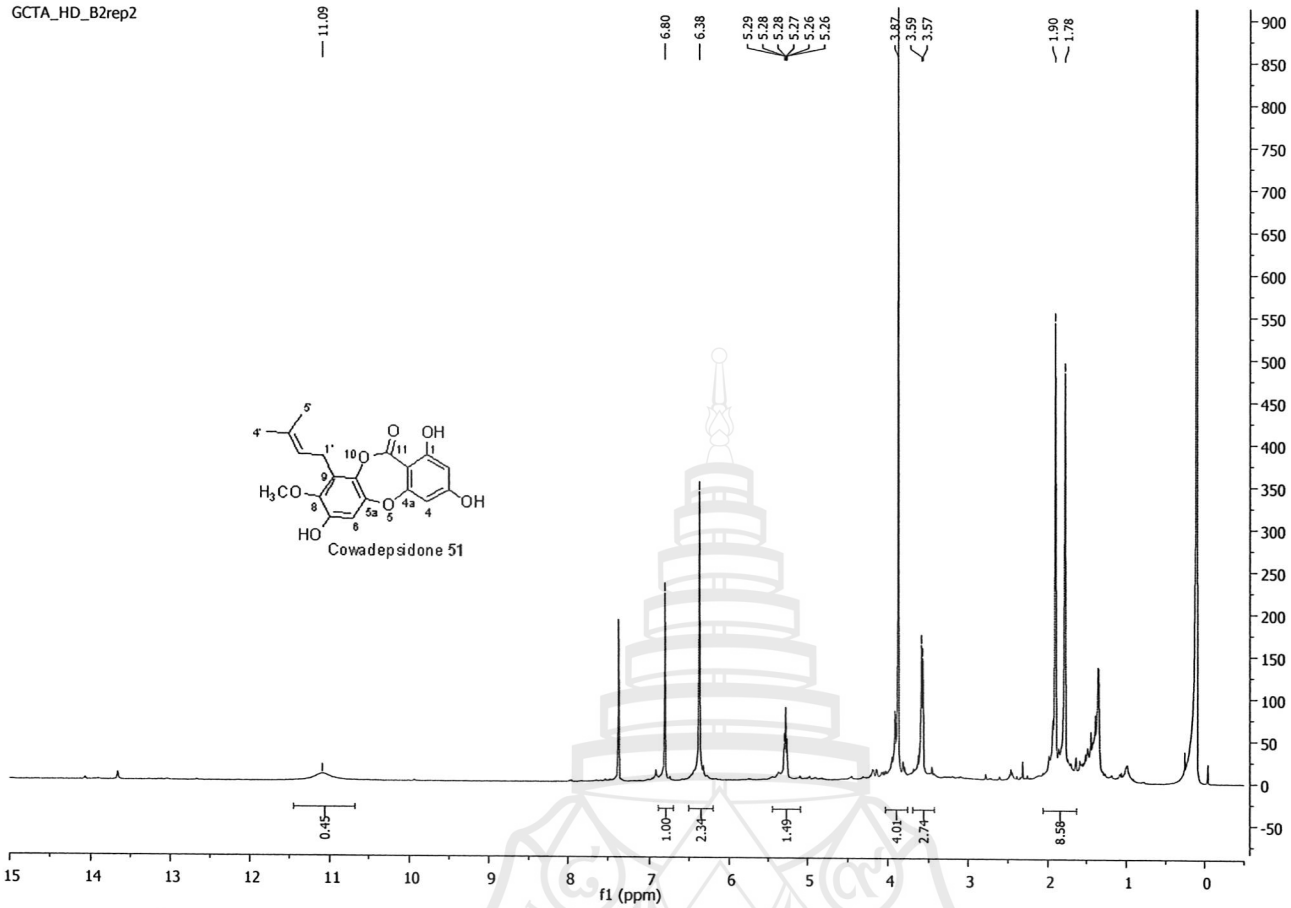


Figure 4-8. ^{1}H NMR of cowadepsidone **(51)**.

Table 4-1. NMR spectral data (400 MHz, $CDCl_3$) of 7 in $CDCl_3$

No.	δ_H (mult., J in Hz)	δ_C	HMBC ($^{1}H \rightarrow {}^{13}C$)
1	10.98 (s)	165.5 (s)	-
2	6.27 (br s)	100.6 (d)*	1, 3, 4, 11a
3	-	163.7 (s)	-
4	6.27 (br s)	100.5 (d)*	2, 3, 4a, 11a
4a	-	161.8 (s)	-
5a	-	146.5 (s)	-
6	6.70 (s)	105.5 (d)	5a, 8, 9a
7	-	147.0 (s)	-
8	-	142.7 (s)	-
9	-	128.3 (s)	-
9a	-	136.0 (s)	-
11	-	168.0 (s)	-
11a	-	98.8 (s)	-
12	3.47 (d, 6.8)	24.1 (t)	8, 9, 9a, 13, 14
13	5.17 (br s)	121.2 (d)	9, 15, 16
14	-	133.2 (s)	
15	1.80 (s)	18.0 (q)	13, 14, 16
16	1.67 (s)	25.7 (q)	13, 14, 15
OMe	3.77 (s)	61.8 (q)	8

* Interchangeable

Compound **7** was isolated as a red gum. Its molecular formula was established as $C_{19}H_{18}O_7$ by ESITOFMS at *m/z* 359.1133 $[M+H]^+$, suggesting the presence of 11 degrees of unsaturations and supported by NMR data (Table 1). The IR spectrum showed hydroxy (3363 cm^{-1}) and lactone carbonyl (1658 cm^{-1}) stretching bands. The presence of the latter functionality was confirmed by resonances at δ 168.0 (C-11) and δ 10.98 (OH-1). The ^1H NMR data of **7** displayed signals of two aromatic protons at δ 6.70 (1H, s, H-6) and 6.27 (2H, br s, H-2, H-4). Furthermore, the proton signals at δ 5.17 (1H, br s, H-13), 3.47 (2H, d, J = 6.8 Hz, H_2-12), 1.80 (3H, s, H_3-15), and 1.67 (3H, s, H_3-16) suggested the presence of a prenyl moiety in the structure.[18] In addition, a methoxyl signal at δ 3.77 (3H, s) was also observed in the ^1H NMR spectrum. Analyzing the 2D NMR spectra using HMQC and HMBC techniques enabled the assignment of ^1H and ^{13}C NMR signals. By comparing the NMR data of **7** with those of the known compound, garcinisidone-A,[19] the possible structure of **7** was established suggesting the same core structure for both compounds. The HMBC correlations of the methoxyl protons at δ 3.77 with the oxygenated carbon at δ 142.7 (C-8) and those of the methylene protons of a prenyl unit at δ 3.47 (H_2-12) with the carbons at δ 142.7 (C-8), 136.0 (C-9a), and 128.3 (C-9) established the attachment of the methoxyl group and the prenyl side chain at C-8 and C-9, respectively. The aromatic proton at δ 6.70 (H-6) showed HMBC connectivity to three aromatic carbons at δ 146.5 (C-5a), 142.7 (C-8) and 136.0 (C-9a), confirming the location of substituents on the B ring. Furthermore, the correlation of aromatic protons at δ 6.27 (H-2, H-4) with the aromatic carbons at δ 165.5 (C-1), 163.7 (C-3), 161.8 (C-4a), 100.6 (C-2), 100.5 (C-4) and 98.8 (C-11a) indicated the orientation of substituents on the A ring. The quaternary carbon signals of δ 165.5 (C-1), 163.7 (C-3), 147.0 (C-7) and its molecular formula $C_{19}H_{18}O_7$ indicated the presence of three hydroxy groups at C-1, C-3 and C-7, respectively. Thus, compound **7** was determined as cowadepsidone which reported for the first time as a metabolite of *G. cowa*.[20]

The biosynthetic analysis of xanthones, originated mainly from a mixed shikimate-acetate pathway, involved the condensation of shikimate and acetate moieties, producing benzophenone intermediate. The xanthone ring formation is derived from regioselectively oxidative mediated intramolecular coupling of benzophenone. Selective installation of hydroxyl group to the aromatic ring of xanthone skeleton is the last step in biosynthetic pathway as shown in Figure 4-9.[21] From the framework of cowadepsidone 51 revealed that its core structure shares xanthone skeleton. It should be implied that the biosynthesis of

depsidone derived from the same pathway as xanthone except the esterification step rather than acylation mechanism.

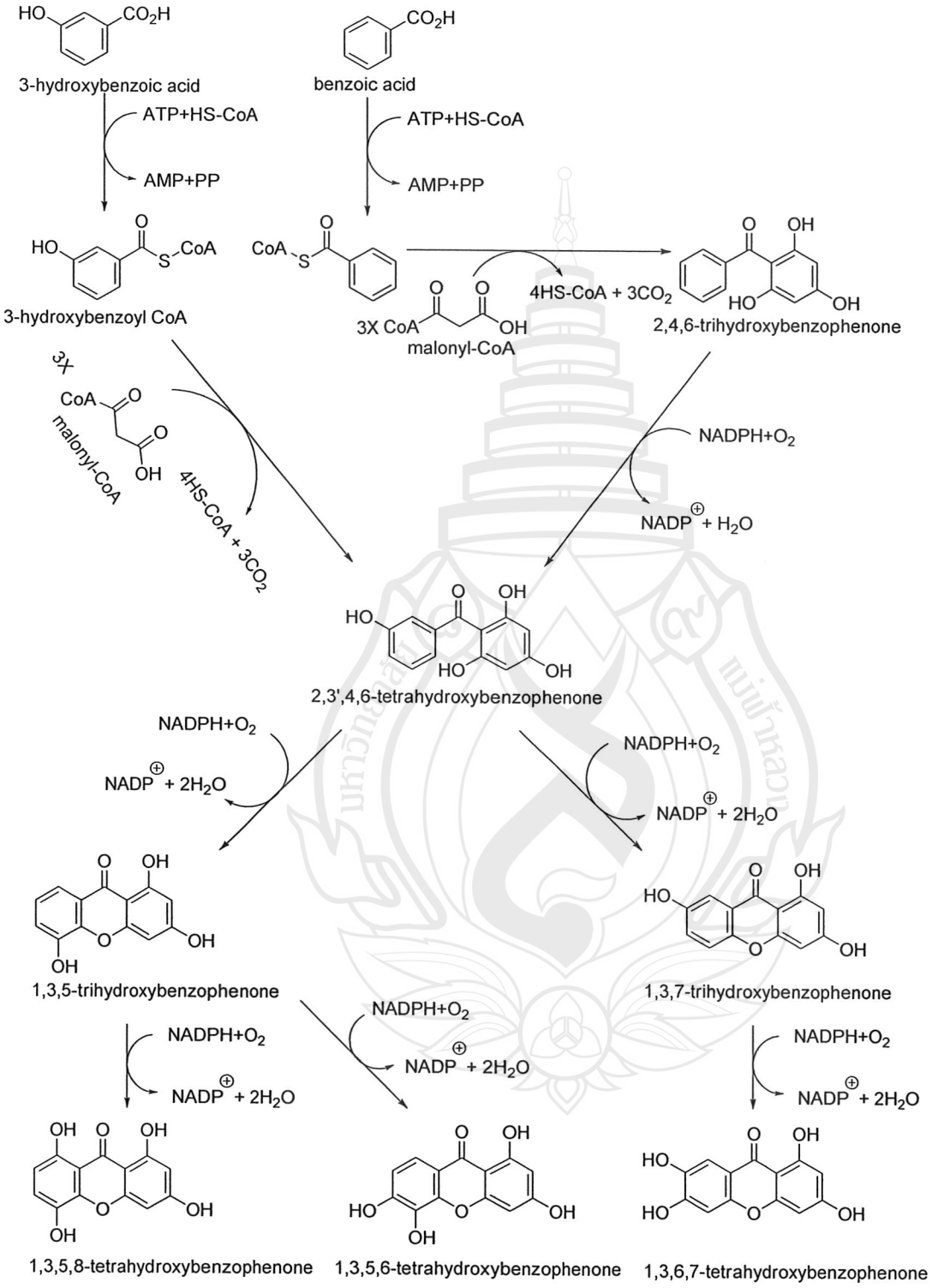


Figure 4-9. Biosynthetic pathway of xanthone.

Table 4-2. Anti-bacterial activity of isolated xanthones **(1)**, **(2)**, **(16)**, **(30)**, **(50)** and depsodone **(51)**.

Compound	MIC (μg/mL)			
	Gram positive		Gram negative	
	MRSA-SK1	S.A	E.C	S.T
1	4	32	128	128
2	2	8	128	128
16	16	128	128	128
30	4	8	64	128
50	64	64	128	128
51	16	16	128	128
Vancomycin	1	0.25	inactive	inactive
Gentamycin	inactive	inactive	0.25	0.125

MRSA-SK1	Methicillin-resistant *Staphylococcus aureus* SK1
S.A	*Staphylococcus aureus* TISTR 1466
E.C	*Escherichia coli* TISTR 780
S.T	*Salmonellae typhimurium* TISTR 292

Almost compounds were further evaluated for their antibacterial activity against Gram negative: *Escherichia coli* TISTR 780, *Salmonellae typhimurium* TISTR 292 and Gram positive: *Staphylococcus aureus* TISTR 1466 (SA) and Methicillin-resistant *S. aureus* (MRSA) SK1. Compounds **1, 2** and **30** showed strong activity against MRSA SK1 with MIC values of 4, 2 and 4 μg/mL, respectively, whereas compounds **16**, **50** and **51** exhibited moderate activity with the MIC value of 16, 64 and 16 μg/mL. Five compounds, **1**, **2**, **30**, **50**, **51**, were also had active against *S. aureus* with the MIC values of 32, 8, 8, 64, 16 μg/mL, respectively. In case of antibacterial activity against Gram negative, *E. coli* and *S. typhimurium*, unfortunately, all of them were found to be weak antibacterial activity against *E. coli* and *S. typhimurium* with the same MIC value of 128 μg/mL except for the antibacterial activity against *E. coli*, compound **30** exhibited moderate activity (MIC value = 64 μg/mL).

While cowanol (**2**) and α-mangostin (**16**) both share isoprenated xanthone, theirs frameworks differ in isoprenyl unit [geranyl group at C-8 in cowanol (**2**) versus isoprenyl group in α-Mangostin (**16**)]. Both of them showed that antibacterial activity against Gram positive bacteria was significant difference. It should be noted that the geranyl group in cowanol (**2**) plays an important role in the antibacterial activity against MRSA SK1 and *S. aureus* as shown in Table 4. Moreover, antibacterial activity against SA of cowanol **(9)** and 1,3,6,7-tetrahydroxyxanthone **(30)** implied that either isoprenol at C-2 or hydroxyl group at C-7 is crucially important for enhancement the antibacterial activity against *S. aureus*.

CHAPTER 5
CONCLUSION

The phytochemical constituents isolated from *G. cowa* are a rich source of xanthones. In this study six known xanthones, cowanin, cowanol, α-mangostin, 1,7-dihydroxyxanthone, 1,3,6,7-tetrahydroxyxanthone, 1,3,7-trihydroxy-2-prenylxanthone and a new cowadepsidone were observed. The antibacterial activity against Gram positive bacteria was significant based on substituents on xanthone aromatic ring. Cowanol and α-mangostin both share isoprenated xanthone, theirs frameworks differ only substituent at C-8, geranyl in cowanol versus isoprenyl group in α-Mangostin. Both of them showed that antibacterial activity against Gram positive bacteria was significantly different.

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