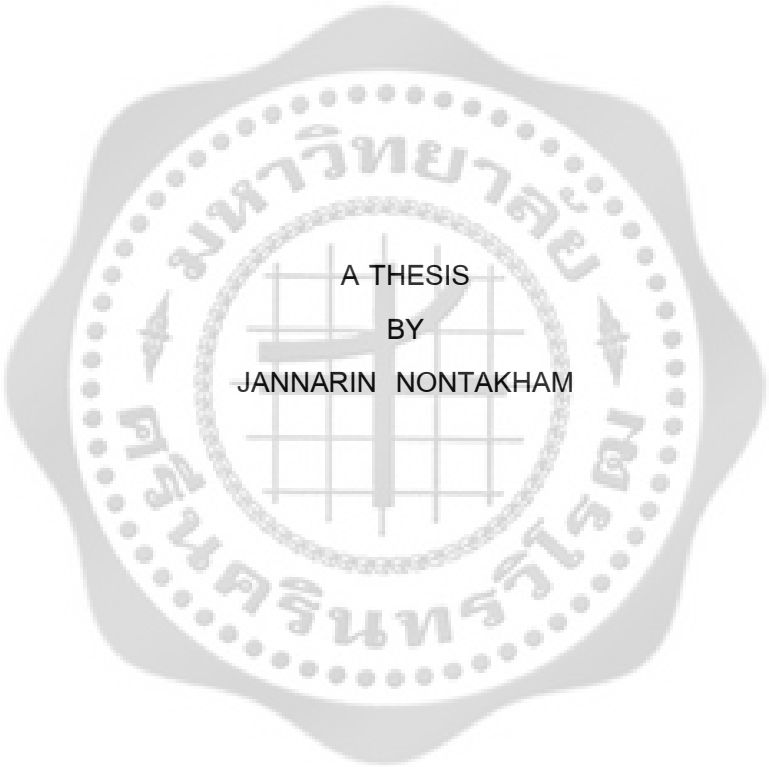
CHEMICAL CONSTITUENTS OF *GARCINIA FUSCA* PIERRE



A THESIS

BY

JANNARIN NONTAKHAM

Presented in Partial Fulfillment of the Requirements for the

Master of Science Degree in Chemistry

at Srinakharinwirot University

May 2011

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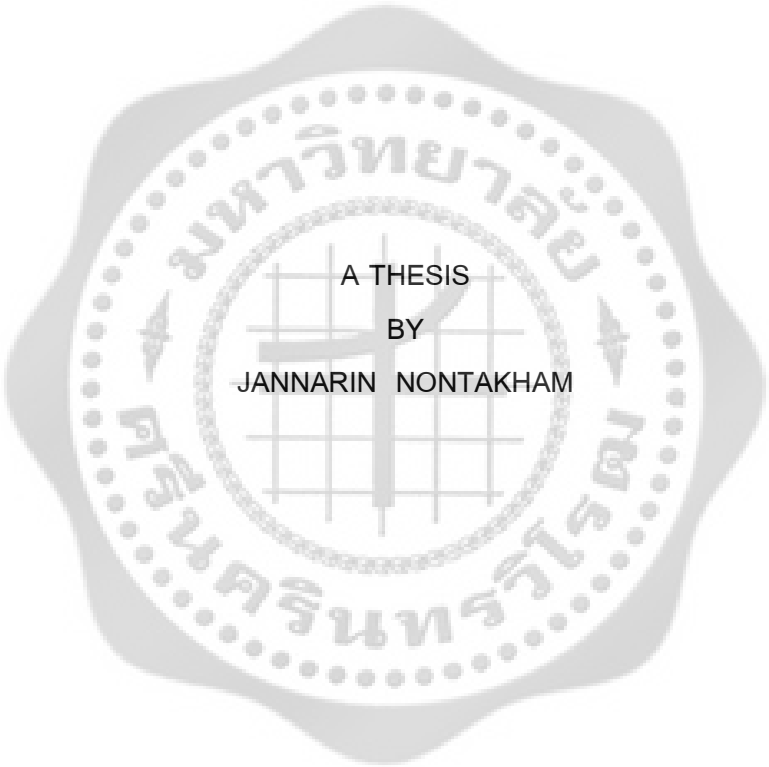

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AN ABSTRACT

BY

JANNARIN NONTAKHAM

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May 2011

Jannarin Nontakham. (2011). Chemical constituents of *Garcinia fusca* Pierre. Thesis, M.Sc. (Chemistry). Bangkok: Graduate School, Srinakharinwirot University. Advisor Committee: Assoc. Prof. Dr. Sunit Suksamrarn, Dr. Prasert Pattanaprateeb.

Garcinia fusca Pierre or “Madan-paa” in Thai (Clusiaceae) is distributed in the North East Thailand. Only one phytochemical study on the stem bark from this plant has been reported. In this investigation, the root and fresh green fruit of *G. fusca* was extracted with organic solvents and the extracts were separated and purified by chromatographic techniques. This led to the isolation of eight known xanthones named α-mangostin (**13**), β-mangostin (**10**), cowanin (**11**), cowaxanthone (**9**), cowanol (**14**), fuscaxanthone G (**7**), 1,3,5,6,-tetrahydroxyxanthone (**17**) and isojacareubin (**18**), together with two known biflavonoids, namely morelloflavone (**19**) and vokensiflavone (**20**), one triterpene named β-sitosterol (**21**) and a mixture of rubraxanthone (**12**) and cowaxanthone (**9**) from the root of this plant. From the fresh green fruit of *G. fusca*, six known xanthones, α-mangostin (**13**), β-mangostin (**10**), cowanin (**11**), cowaxanthone (**9**), cowanol (**14**) and fuscaxanthone A (**1**) were isolated. This is the first report on isolation of compounds **17**-**20** from this plant. The structures of all compounds were elucidated by spectroscopic techniques, especially 1D and 2D NMR and MS including by comparison of their spectroscopic data with those reported in the literature.

องค์ประกอบทางเคมีของต้นมะดันป่า

บทคัดย่อ

ของ

จันทร์นรินทร์ นนทะขาม

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา

ตามหลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาเคมี

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จันทร์นรินทร์ นนทะขาม. (2554). *องค์ประกอบทางเคมีของต้นมะดันป่า*. ปริญญานิพนธ์ วท.ม. (เคมี). กรุงเทพฯ: บัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ. คณะกรรมการควบคุม: รองศาสตราจารย์ ดร.สุนิตย์ สุขสำราญ, ดร. ประเสริฐ พัฒนาประทีป.

มะดันป่า (*G. fusca* Pierre.) เป็นพืชในวงศ์ Clusiaceae พบในแถบภาคตะวันออกเฉียงเหนือ ของประเทศไทย จากรายงานวิจัยที่เกี่ยวข้องมีเพียงหนึ่งรายงานการวิจัยเท่านั้นที่ทำการศึกษาองค์ประกอบทางเคมีของส่วนเปลือกต้น ในงานวิจัยนี้ได้ทำการศึกษาองค์ประกอบทางเคมีของส่วนรากและผลดิบสดจากมะดันป่า เมื่อนำส่วนรากและผลดิบสดมาสกัดด้วยตัวทำละลายอินทรีย์ และนำสารสกัดมาแยกและทำให้บริสุทธิ์ด้วยเทคนิคโครมาโทกราฟีชนิดต่างๆ พบว่าสามารถแยกสารแซนโทนจากส่วนรากได้ 8 ชนิด ได้แก่ α-mangostin (**13**), β-mangostin (**10**), cowanin (**11**), cowaxanthone (**9**), cowanol (**14**), fuscaxanthone G (**7**), 1,3,5,6,-tetrahydroxyxanthone (**17**), isojacareubin (**18**) พบสารไบฟลาโวนอยด์ 2 ชนิดได้แก่ morelloflavone (**19**) และ vokensiflavone (**20**), สารกลุ่มไตรเทอพีน 1 ชนิด คือ β-sitosterol (**21**) และได้สารแซนโทนผสมระหว่าง cowaxanthone (**9**) และ rubraxanthone (**12**) อีกทั้งสามารถแยกสารแซนโทนจากส่วนผลดิบสดได้ 6 ชนิด ได้แก่ α-mangostin (**13**), β-mangostin (**10**), cowanin (**11**), cowaxanthone (**9**), cowanol (**14**) และ fuscaxanthone A (**1**) รายงานนี้เป็นการรายงานครั้งแรกของการแยกสาร **17**-**20** จากพืชชนิดนี้ การพิสูจน์โครงสร้างของสารบริสุทธิ์ใช้เทคนิคทางสเปกโทรสโกปี โดยเฉพาะอย่างยิ่ง 1D และ 2D นิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโคปี และแมสสเปกโทรมิเตอร์ รวมทั้งโดยการเปรียบเทียบข้อมูลกับที่มีผู้รายงานไว้แล้ว

The thesis titled

"Chemical constituents of *Garcinia fusca* Pierre"

by

Jannarin Nontakham

has been approved by the Graduate School as partial fulfillment of the requirements for the

Master of Science Degree in Chemistry of Srinakharinwirot University.

..... Dean of Graduate School

(Assoc. Prof. Dr. Somchai Santiwattanakul)

May 27, 2011

Thesis Committee

..... Major advisor

(Assoc. Prof. Dr. Sunit Suksamrarn)

..... Co-advisor

(Dr. Prasert Pattanaprateeb)

Oral Defense Committee

..... Chair

(Prof. Dr. Apichart Suksamrarn)

..... Committee

(Assoc. Prof. Dr. Sunit Suksamrarn)

..... Committee

(Asst. Prof. Dr. Siritron Samosorn)

..... Committee

(Dr. Prasert Pattanaprateeb)

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CHAPTER 1

INTRODUCTION

Background

Garcinia, belongs to the family Clusiaceae, is the best known in Malaysia as a genus of fruit trees (Peres; et al. 2000: 683-710). There are about 450 species distributed in tropical and South Africa, Madagascar, tropical Asia, North East Australia, West Polynesia, tropical America and 20 species in China (Xiwen; et al. 2007, 13: 40–47). The fruit of most species in this genus are edible, among them, those of *G. mangostana* are famous. The seed yields more than 15% oil. The yellow resin of some species is used as a medicine. Species like *G. hanburyi* J. D. Hooker provide medicinal resin and yellow dyes of the best quality. The timber of many species is used for building houses or making furniture (Xiwen; et al. 2007, 13: 40–47).

Extracts of *Garcinia* species is a rich source of oxygenase and prenylated xanthones. These xanthones have been shown various bioactivities for example antimethicillin-resistant *Staphylococcus aureus* (MRSA) (Rukachaisirikul; et al. 2003: 933-938, Rukachaisirikul; et al. 2005: 165-170 and Sukpondma; et al. 2005: 850-852), antivancomycin resistant *Enterococci* (VRE) (Sakagami; et al. 2005: 203-208), antimicobacterial activity (Suksamrarn; et al 2003: 857-859), antimalarial (Ignatushchenko; et al. 2000: 77-81), tumor-promoting inhibition (Ito; et al. 2003: 200-205), selective cyclooxygenase-2 inhibition (Zou; et al. 2005: 1514-1518), inhibitory effects on PAF-induced hypotension (Oku; et al. 2005: 90-92), antibacterial activity (Rukachaisirikul; et al. 2000: 8539-8543), antimalarial activity (Likhitwitayawuid; et al. 1998: 237-241), cytotoxicity (Suksamrarn; et al. 2006: 301-305), anticancer (Matsumoto; et al. 2003: 1124-1127), antifungal (Garcia; et al. 1998: 1367-1374), antiinflammatory (Nakatani; et al. 2004: 667-674) and antioxidant properties (Lee; et al. 2005: 5548-5552).

*Garcinia*s in Thailand

There are 23 species of *Garcinia* in Thailand (Smitinand. 2001: 158-159), as follows

1 *G. acuminate* Planch. & Triana or Rong thong in Nakhon Si Thammarat (รงทอง)

2 *G. atroviridis* Griff. or Som khaek in Pattani (ส้มแขก)

3 *G. costrata* Hemsl. or Mangkhut paa in Satun (มังคุดป่า)

4 *G. cowa* Roxb. or Cha muang in Central (ชะมวง)

5 *G. dulcis* Kurz or Ma phuut in Pattani (มังพูด)

6 G. *elliptica* Wall. or *G. acuminate* Planch. & Triana.

7 *G. fusca* Pierre or Madan paa in Maha Sarakham (มะดันป่า)

8 *G. gracilis* Pierre or Mak paem in Nong Khai (หมากแปม)

9 *G. hanburi* Hookf. f. or Rong in Chantabury and Trat (รง)

10 *G. hombroniana* Pierre or Waa in Yala (วา)

11 *G. lanessanii* Pierre or Somkung yai in Khon Kaen (ส้มกุ้งใหญ่)

12 *G. mackeaniana* Craib or Mada in Phrae (มะดะ)

13 *G. mangostana* L. or Mangkhut in General (มังคุด)

14 *G. merguensis* Wight or Nuan in Northern (นวล)

15 *G. nervosa* Miq. or Maphut paa in Pattani (มะพูดป่า)

16 *G. nigrolineata* Planch. or Chamuang in Trat (ชะมวง)

17 *G. rostrata* Benth. & Hook. f. or Muang laai in Surat Thani (ม่วงลาย)

18 *G. schomburgkiana* Pierre or Madan in Central (มะดัน)

19 *G. speciosa* Wall. or Phawa in Surat Thani (พะวา)

20 *G. succifolia* Kurz or Mapong ton in Northern (มะป่องต้น)

21 *G. thorelii* Pierre or Mada kheenon in Chiang Rai (มะดะขี้หนอน)

22 *G. vilersiana* Pierre or Phawaa baiyai in Chon Buri and Chanthabury (พะวาใบใหญ่)

23 *G. xanthochymus* Hook. f. or Mada luang in Chiang Mai (มะดะหลวง)

Botanical Description of *Garcinia fusca*

Garcinia fusca, known in Thai as “Madan-paa” or Mak-Mong, is similar to *G. subfalcata*, but the latter differs in having more numerous secondary leaf veins (in 28-32 pairs), staminodes united into 4 bundles, and stigma with papillae arranged in pairs (Xiwen; et al. 2007, 13: 40–47). *G. subfalcata* is an erect slow-growing tree about 7 m tall, about 15 cm in diameter with dark brown bark. A branch striate and twigs with broken rings. Petiole 0.4–1.2 cm. Leaf blade narrowly elliptic or elliptic-lanceolate, 3.5–8 × 0.8–2.5 cm, papery, midvein raised abaxially, flat adaxially; secondary veins 7–13 pairs, near margin arching and anastomosing, tertiary veins sparse, inconspicuous, base attenuate, slightly decurrent, apex long acuminate, usually falcate, rarely obtuse. Plant dioecious. Female flowers solitary or in pairs, usually at apex of branchlet, sometimes axillary; pedicels about 2 mm. There are four sepals as two outer: suborbicular, short and two inner: narrowly elliptic, thicker and four petals, nearly equal, oblong, and slightly longer than sepals, about 5 mm. There are four staminodes; anthers 4-celled; cells longitudinally dehiscent; connectives thickened; filaments robust, about 1 mm; ovary ovoid, sulcate outside; style nearly absent; the stigma radiately lobed, papillate. The fruit is globose, about 3 cm in diamiter, smooth, nearly sessile.

FIGURE 1 Pictures of *G. fusca* tree and their fruit.

Ethanopharmacological Uses of *Garcinia* Plants

G. dulcis grows mainly in Southeast Asia, and its leave and seed have been used in traditional medicine against lymphatitis, parotitis, struma and other disease conditions (Iinuma; et al. 1996: 1195-1196). The oil obtained from the seed of *G. echinocarpa* is used for lighting lamps (Bandaranayake; et al. 1975: 1878-1880). *G. livingstonei* is a small to medium-sized tree producing edible fruits and growing at low altitude. It is found, particularly in South Africa, in riverine fringes and in open woodland. Extracts of the leaves and flowers are reported to exhibit antibiotic properties (Diserens; et al. 1992: 313-316). The fruit hull of *G. mangostana* L., the "Mangosteen" tree, is used in Thai folk medicine for healing skin infections and wounds, and for the relief of diarrhea (Mahabusarakam; Wiriyachitha; & Taylor.; et al. 1987:474-478). It is fairly widespread in India, Sri Lanka and Burma. In the Ayurvedic system of medicine, the fruit hull of this plant finds wide application, mainly as an anti-inflammatory agent and in the treatment of diarrhea (Balasubramanian; & Rajagopapan. 1988: 1552-1554). *G. subelliptica* is a small shrub 4-5 m in high or a large tree sometimes reaching 15-20 m, and has been extensively cultivated as a windbreak in the Yaeyama islands of Japan. Its bark has been utilized as a source of a yellow coloured dye (Fukuyama; et al. 1991: 3433-3436).

G. fusca is distributed in the North East of Thailand. Young leaves are eaten as vegetables either raw or in curry. Ripe fruit are edible but acidic, used to make a refreshing drink. The root and leaves are used for relief coughs and fever. Barks may be boiled in water to remedy for fever and skin disease.

From the reports on the biological activities of *Garcinia* plants, together with only one study on phytochemicals of *G. fusca* stem bark has been reported (Ito; et al. 2003: 200-5). It is therefore interesting to study the chemical constituents of other parts of this plant. Thus, the root and fresh green fruit of *G. fusca* are selected for this study to search for xanthones and other constituents.

Objectives of the study

1. To isolate and purify components from the air dried root and fresh green fruit of *G. fusca* Pierre.

2. To determine chemical structures of the isolated compounds.

CHAPTER 2

REVIEW OF LITERATURES

As the quest for new natural products continues, it becomes increasingly clear that xanthones are very restricted in occurrence. The majority of natural xanthones have been found in just two families of higher plants Guttiferae and Gentianaceae simple, oxygenated xanthones occur in both families and are generally more highly oxygenated in the Gentianaceae. Prenylated xanthones are widely distributed in the Guttiferae but not known in the Gentianaceae, and whereas *O*-glycosylxanthones are common in the Gentianaceae, only two have been reported from the Guttiferae (Graham; & Lee. 1989: 967-998).

Several earlier reviews have summarized the literature on xanthones, with emphasis on biosynthesis, synthesis or phylogeny. In 1980, Sultanbawa listed 95 xanthones from the Clusiaceae. Since then there has been a steady stream of reports in which more than 80 new xanthones have been characterized and many known xanthones re-isolated from about 60 species of Guttiferae (Graham; & Lee. 1989: 967-998). In 2010, Chantarasriwong summarizes the explorations of the caged *Garcinia* xanthones, a family of plant metabolites that possess a unique chemical structure, potent bioactivities, and a promising pharmacology for drug design and development.

The symmetrical nature of the xanthone nucleus, coupled with its mixed biogenetic origin in higher plants, necessitates that the carbons be numbered according to a biosynthetic convention. Carbons 1-4 are assigned to the acetate-derived ring A and carbons 5-8 to the shikimate-derived ring B (Figure 2) (Graham; & Lee. 1989: 967-998).

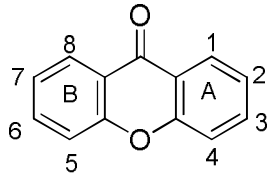


FIGURE 2 Structure of xanthone.

Chemical constituents of *G. fusca*

In 2003, Ito; et al. found eight new xanthones, fuscaxanthones A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**) and H (**8**), together with eight known xanthones, namely cowaxanthone (**9**), β-mangostin (**10**), cowanin (**11**), rubraxanthone (**12**), α-mangostin (**13**), cowanol (**14**), norcowanin (**15**) and 7-*O*-methylgarcinone E (**16**) from acetone extract of the stem bark of *G. fusca* collected in Thailand (Ito; et al. 2003: 200-205).

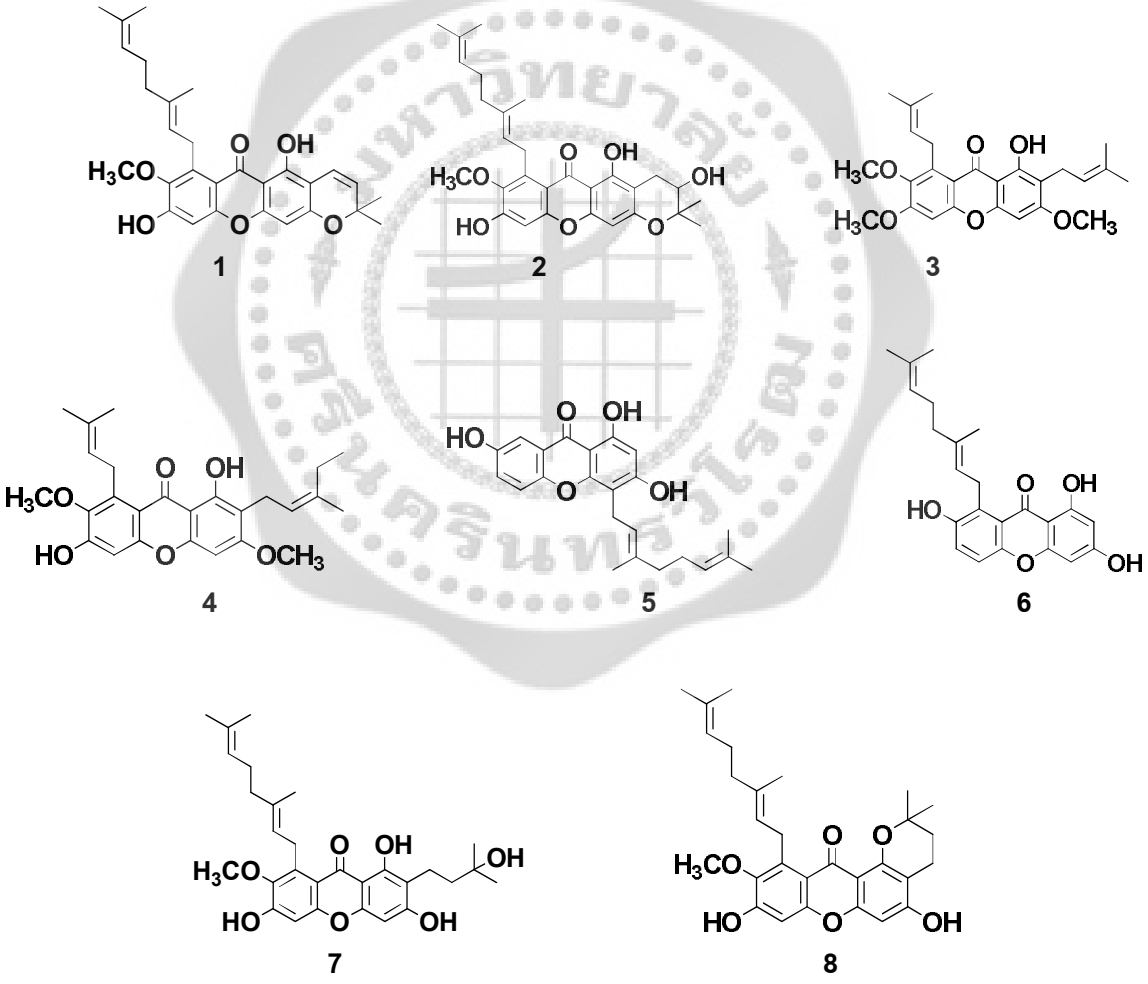


FIGURE 3 Structures of xanthones **1**-**8** from *G. fusca*

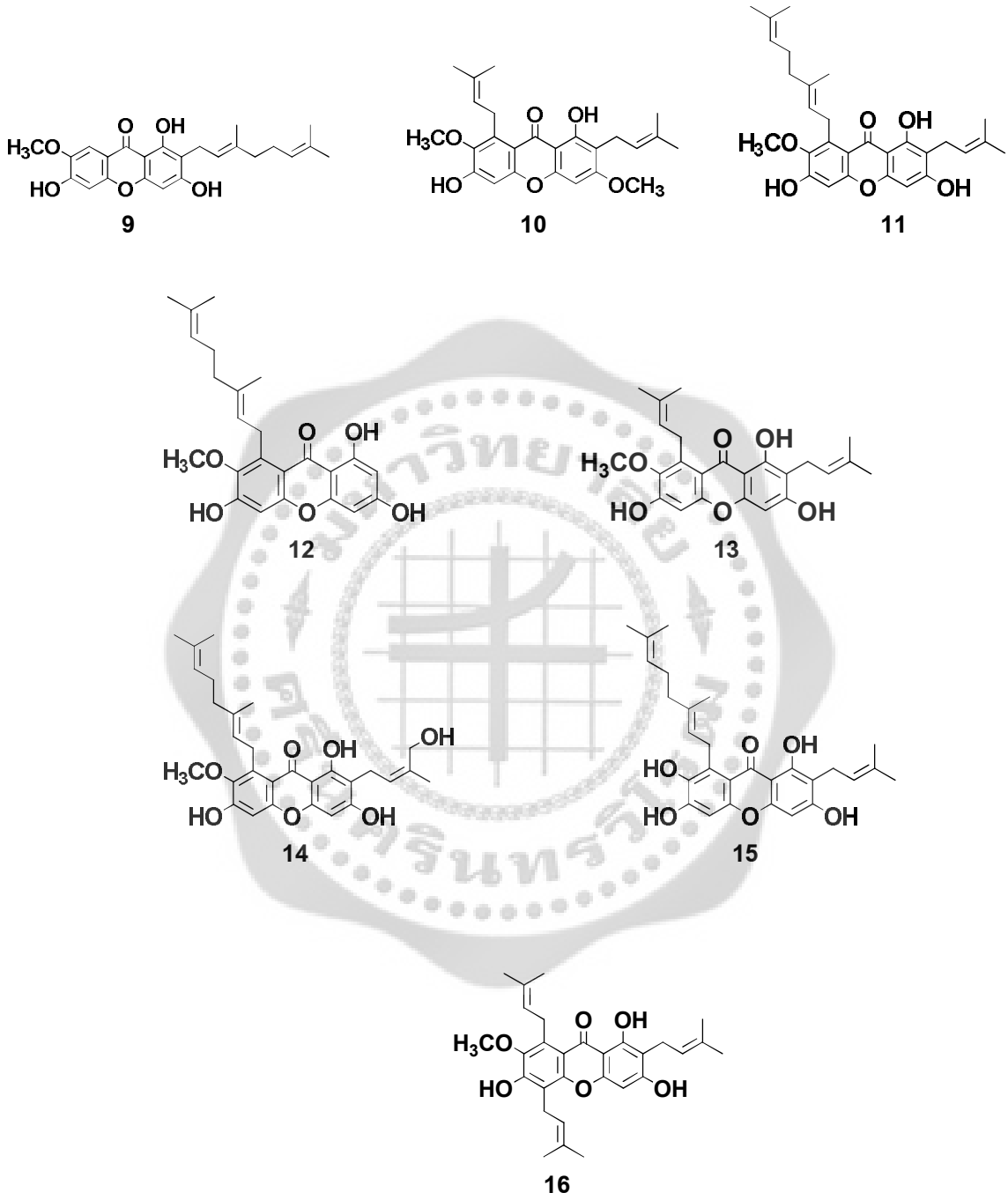


FIGURE 4 Structures of xanthones **9-16** from *G. fusca*

Furthermore, in a primary screening test for novel cancer chemopreventive agents (anti-tumor promoters), they found that several xanthones and depsidones showed potent inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. In the course of them continuing search for active cancer chemopreventive compounds from higher plants, they also carried out a primary screening of eight known xanthones that isolated in this study by examining their possible inhibitory effects on EBV-EA activation.

Eight xanthones (**9**-**16**) isolated as major components of *G. fusca* were tested for their tumor-promoting inhibitory activity by using as short-term *in vitro* assay of TPA-induced EBV-EA activation in Raji cells. Their inhibitory effects on the activation of the virus-genome and the viability of Raji cells and the 50% inhibitory concentration IC_{50} values are shown in Table 1. All the test compounds showed inhibitory activity of EBV-EA activation even at 1x10 mol ratio/TPA (2.5-16.2%) and fully blocked EBV-EA activation at high concentration ($1x10^3$ mol ratio/TPA) without causing a decrease in viability (>70%) of the raji cells. The corresponding IC_{50} values of tested compounds were within the range of 210-398 mol ratio/TPA and were lower than that of β-carotene (IC_{50} 400), a vitamin A precursor commonly used in cancer prevention studies. Of the other compounds, 7-*O*-methylgarcinone (**16**), having three prenyl side chains at C-2, C-5, and C-8 of the xanthone nucleus, exhibited the most potent inhibitory activity (IC_{50} 210; 100, 83.7, 40.8, and 16.2% inhibition of activation at 1000, 500, 100 mol ratio/TPA, respectively). Furthermore, β-mangostin (**10**) and α-mangostin (**13**), having two prenyl side chain at both C-2 and C-8 of the xanthone nucleus, showed significant inhibitory activity (IC_{50} 270 and 220 mol ratio/TPA TPA, respectively). The inhibitory activity of cowanin (**11**), cowanol (**14**), and norcowanin (**15**), replacing the C-5 side chain (prenyl group) with a C-10 side chain (geranyl group) at C-8, was weaker (IC_{50} 310-320 mol ratio/TPA) than that of compounds **10**, **13**, and **16**. The corresponding IC_{50} values of cowaxanthone (**9**) and rubraxanthone (**12**), with only one geranyl group at C-2 or C-8 in the molecule, were 389 and 340 mol ratio/TPA, respectively. From the viewpoint of structure-activity relationship, an essential feature for the activity of the xanthones examined in the present study is the presence of the two C-5 side chains (prenyl group) at the 2- and 8-positions in a xanthone skeleton that has oxygen-linked substituent at positions 1,3,6 and 7. In previous studies, Ito et al. reported that the presence of a prenyl moiety in the 1,3-dihydroxyxanthone molecular plays an important role in producing inhibitory

effects on EBV-EA induction. In view of the present findings taken together, the relative location of a hydroxyl group and a hydrophobic prenyl moiety on the xanthone nucleus might be important factors in producing the observed chemoproventive effect against chemical induced carcinogenesis activity of these compounds in vitro is now in progress (Ito; et al. 2002: 200-205).

TABLE 1. Inhibitory effects of xanthones on TPA-Induced EBV-EA activation[a] (Ito; et al. 2002: 200-205).

compounds	EBV-EA-positive cell (% viability)				IC_{50}[b]
	Compound concentration (mol ratio/32 pmol TPA)				
	1000	500	100	10	(mol ratio/32 pmol TPA)
cowaxanthone (**9**)	0.0±0.4(70)	40.6±1.8(>80)	76.6±2.2(>80)	97.5±0.7(>80)	398
β-mangostin (**10**)	0.0±0.3(70)	20.5±1.4(>80)	62.6±2.4(>80)	90.4±0.7(>80)	270
cowanin (**11**)	0.0±0.4(70)	33.6±1.8(>80)	71.6±1.2(>80)	95.8±0.7(>80)	320
rubraxanthone A (**12**)	0.0±0.3(70)	39.5±1.2(>80)	74.1±2.5(>80)	96.8±0.5(>80)	340
α-mangostin (**13**)	0.0±0.5(70)	19.1±1.1(>80)	60.0±2.2(>80)	89.2±0.3(>80)	220
cowanol (**14**)	0.0±0.3(70)	30.5±1.3(>80)	69.6±2.5(>80)	93.1±0.5(>80)	310
norcowanin (**15**)	0.0±0.5(70)	31.9±1.4(>80)	70.1±1.9(>80)	94.2±0.3(>80)	315
7-*O*-methylgarcinone E (**16**)	0.0±0.8(70)	16.3±1.1(>80)	59.2±1.9(>80)	83.8±0.9(>80)	210
β-carotene[c]	0.0±0.5(70)	34.3±1.1(>80)	82.7±1.8(>80)	100.0±0.2(>80)	400

[a] Mol ratio/TPA (32pmol = 20 ng/mL), 1000 molratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol. Values are EBV-EA activation (%) ±SD in the presence of the test compound relative to the positive control (100%). Values in parentheses represent the surviving Raji cells measured with Trypan Blue staining. At least 60% surviving Raji cells 2 days after treatment with the compounds is required for an accurate result.

[b] IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA.

[c] Positive control substance.

TABLE 2 Bioactivities of isolated xanthones from *G. fusca*

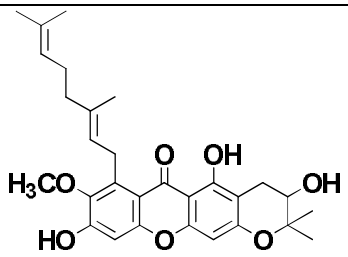
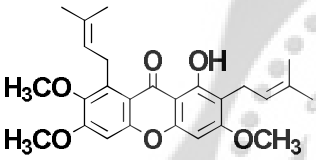
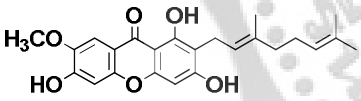
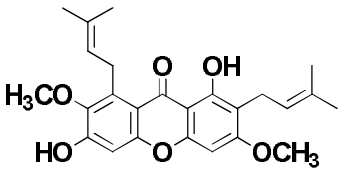
Compounds	Bioactivities	References
 fuscaxanthone A (**1**)	Radical scavenging activity	Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153
 fuscaxanthone C (**3**)	Antibacterial activities	Panthong; et al. 2006: 999-1004
 cowaxanthone (**9**)	Tumor-promoting inhibition Radical scavenging activity Antibacterial activities Cytotoxic activities	Ito; et al. 2003: 200-205 Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153 Panthong ; et al. 2006: 999-1004 Ha; et al. 2009 : 830-834
 β-mangostin (**10**)	Tumor-promoting inhibition Antitumor promotive Antibacterial activities	Ito; et al. 2003: 200-205 Sakai; et al. 1993: 958-960 Panthong; et al. 2006: 999-1004 and Mahabusarakam; Wiriyachitra; & Phongpaichit. 1986: 239-242

TABLE 2 (continued)

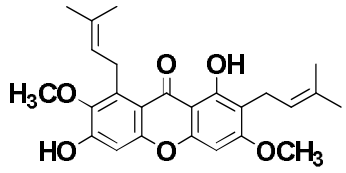
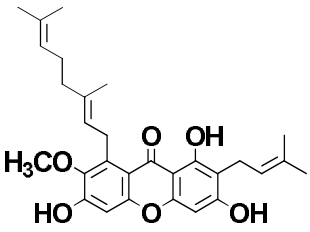
Compounds	Bioactivities	References
	Cytotoxic activities	Suksamrarn; et al. 2006: 301-305
	Antimicrobial	Sundaram; et al. 1983: 59-60
	Antimycobacterial	Suksamrarn; et al. 2003: 857-859
β-mangostin (**10**) (continued)	Inhibit $Ca2^{+}$ dependent protein kinase	Jinsart; et al. 1992: 3711-3713
	Against cAMP phosphodiesterase	Chairungsrilerd; et al. 1996: 1099-1102
	Histaminergic & serotonergic receptor blocking substances	Chairungsrilerd; et al. 1996: 471-472
	Against HIV-1 protease	Chen; Wan; & Loh. 1996: 381-382
	Against DNA topoisomerase I & II	Tosa; et al. 1997: 418-420
	Antifungal activities	Gopalakrishnan; Banumathi; & Suresh. 1997: 519-524
	Induction of apoptosis in human leukemia cell lines	Matsumoto; et al. 2003: 1124-1127
	Aromatase Inhibitory Activity	Itoh; et al. 2008: 4500-4508
 cowanin (**11**)	Tumor-promoting inhibition	Ito; et al. 2003: 200-205
	Radical scavenging activity	Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153
	Antibacterial activities	Panthong; et al. 2006: 999-1004
	Cytotoxic activities	Ha ; et al. 2009 : 830-834

TABLE 2 (continued)

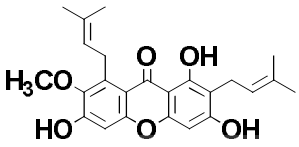
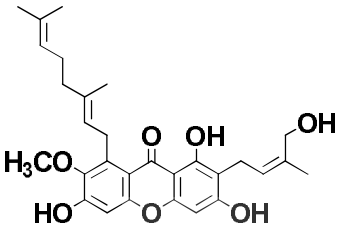
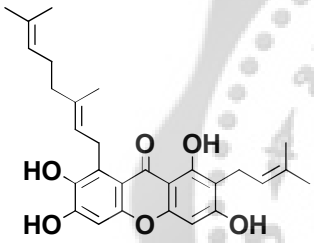
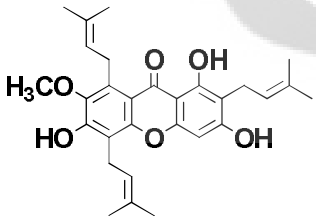
Compounds	Bioactivities	References
 α-mangostin (**13**)	Tumor-promoting inhibition	Ito; et al. 2003: 200-205
	Antibacterial activities	Panthong; et al. 2006: 999-1004
	Antimycobacterial	Suksamrarn; et al. 2003: 857-859
	Antiplasmodial	Azebaze; et al. 2006: 111-113
	Cytotoxic activities	Ha; et al. 2009 : 830-834, Kijjoa; et al. 2008: 864-866 and Suksamrarn; et al. 2006: 301-305
	Antioxidant	Jung; et al. 2006: 2077-2082
	Antimicrobial	Sundaram; et al. 1983: 59-60
	Inhibit Ca^{2+} dependent protein kinase	Jinsart; et al. 1992: 3711-3713
	Against cAMP phosphodiesterase	Chairungsrilerd; et al. 1996: 1099-1102
	Histaminergic & serotonergic receptor blocking substances	Chairungsrilerd; et al. 1996: 471-472
	Against HIV-1 protease	Chen; Wan; & Loh. 1996: 381-382
	Against DNA topoisomerase I & II	Tosa; et al. 1997: 418-420
	Induction of apoptosis in human leukemia cell lines	Matsumoto; et al. 2003: 1124-1127
	Aromatase inhibitory activity.	Itoh; et al. 2008: 4500-4508

TABLE 2 (continued)

Compounds	Bioactivities	References
 cowanol (**14**)	Tumor-promoting inhibition Radical scavenging activity Antibacterial activities Cytotoxic activities	Ito; et al. 2003: 200-205 Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153 Panthong; et al. 2006: 999-1004 Ha; et al. 2009 : 830-834
 norcowanin (**15**)	Tumor-promoting inhibition	Ito; et al. 2003: 200-205
 7-*O*-methylgarcinone E (**16**)	Tumor-promoting inhibition	Ito; et al. 2003: 200-205

CHAPTER 3

EXPERIMENTAL

Source of plant material

The fresh green fruit and the root of *G. fusca* were collected from Buayai Subdistrict, Nampong District, Khon Kaen Province, Thailand, in April 2008 and July 2009, respectively. A voucher specimen (Jannarin Nontakham 001) has been deposited at the Chemistry department of Srinakharinwirot University and was identified by Mr. James F. Maxwell, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

General techniques

1. Thin-Layer chromatography (TLC)

Technique: One dimension, ascending

Adsorbent: Silica gel 60 GF_{254} precoated on aluminium plate (Merck 1.05554)

Layer Thickness: 1.25 mm

Plate size: 1 x 5 cm and 2 x 5 cm

Detection: 1. Spots on TLC were visualized under ultraviolet light at wavelengths of 254 and 365 nm.

2. Developing agent. Anisaldehyde-sulphuric reagent (2.5% v/v in absoluted methanol containing 3.4% v/v sulphuric acid and 1.0% v/v glacial acetic acid). After heating of TLC plate at 100-110 ^{o}C for 1-2 minutes, the spots of organic compounds will give specific colors with this reagent.

2. Column chromatography (CC)

2.1 Liquid column chromatography

Absorbent: 1. Silica gel 60 particle size < 0.063 mm (Merck 1.07729)

2. Silica gel 60 particle size 0.040-0.063 mm (Merck 1.09375)

Packing method: Slurry packing method

Sample loading: The sample will be dissolved in a small volume of suitable organic solvent. The solution will be mixed with silica gel particle size < 0.063 mm or 0.040-0.063 mm. The sample will be evaporated under reduced pressure and added onto the top of column.

Elution: After loading of sample onto the column and appropriate solvent system will be used as a mobile phase in the isocratic or gradient systems.

2.2 Quick column chromatography

Adsorbent: Silica gel 60 GF_{254} for thin-layer chromatography (Merck 1.07730)

Packing method: Dry vacuum packing method

Sample loading: The sample was dissolved in a small volume of an appropriate solvent. The solution was mixed with Merck silica gel 60 GF_{254}. The sample was evaporated under reduced pressure and added onto the top of column.

Elution: After loading of sample onto the column, an appropriate solvent system was used as a mobile phase in the gradient systems.

2.3 Size-Exclusion gel column chromatography

Adsorbent: Sephadex-LH 20

Packing method: Slurry packing method

Sample loading: The sample was dissolved in a small volume of MeOH and added onto the top of column.

Elution: The column was eluted with MeOH.

3. Centrifugal Thin-layer chromatography

Absorbent: Silica gel 60 PF_{254} containing $CaSO_4$ (Merck 1.07749)

Packing method: Sorbent layers on rotors are produced by casting sorbent-binder mixtures followed by scraping down to 1 mm, 2 mm or 4 mm thickness with a roting scraping tool. Before use rotor coated, it should be dried in oven at 70°C 1 hour for activation of chromatotron rotor.

Sample loading: Dissolved the sample in a small volume (0.5-1 ml) of ethyl acetate or methanol. Turn on the rotor and introduce the sample solution with a dropper or syringe into solvent inlet. Allow a few minutes for solvent to drain from the rotor.

Elution: After loading of sample into the chromatotron, an appropriate solvent system will be used as a mobile phase in the isocratic or gradient systems.

Physical Property

Melting points

Melting points were measured on Griffin melting point apparatus in degree Celsius of temperature.

Spectroscopy

1. Infrared (IR) Absorption Spectra

IR spectra were measured on Perkin Elmer FT-IR spectrum BX spectrometer by using potassium bromide (KBr) disc.

2. Ultraviolet (UV) Absorption Spectra

UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer.

3. Mass Spectra

Electrospray ionization mass spectra (ESIMS) were measured on Finnigan LC-Q mass spectrometer.

4. Nuclear Magnetic Resonance (NMR) Spectra

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were determined on a Bruker Avance 300 FT-NMR spectrometer.

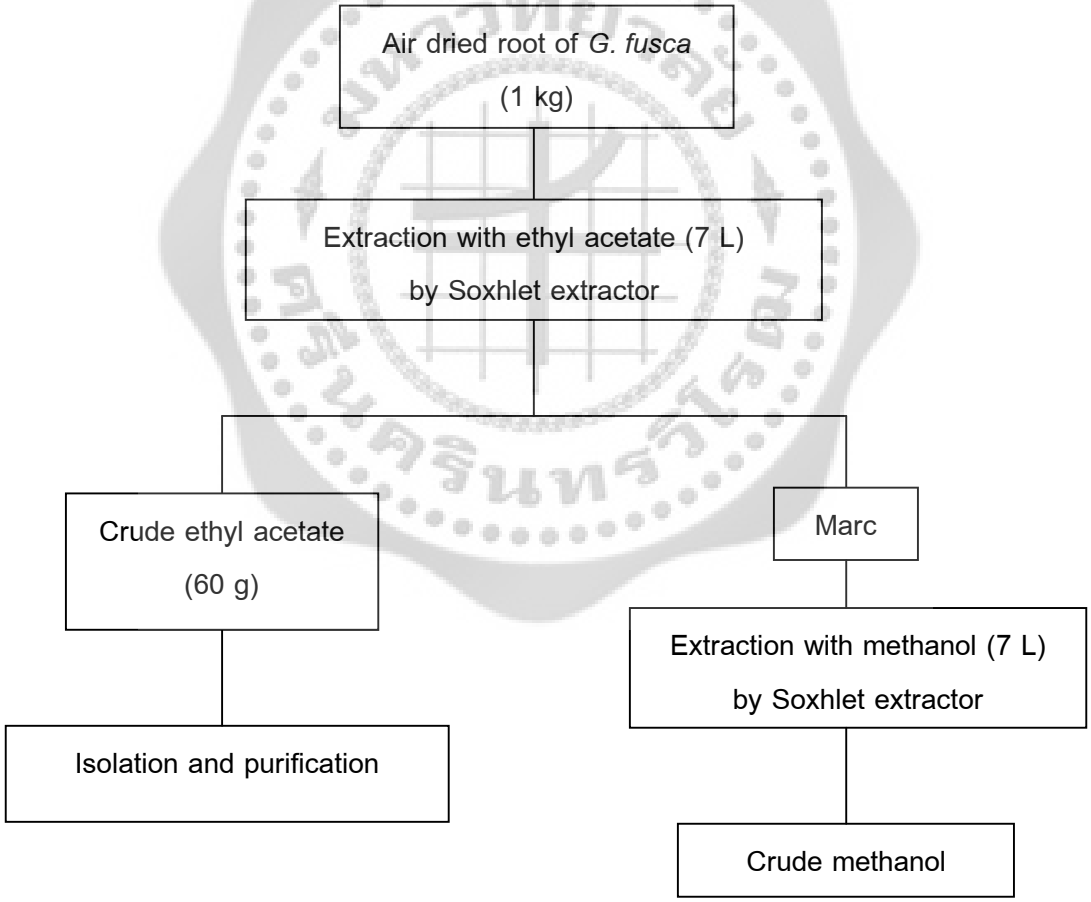
Extraction and Isolation

1. Extraction of the root of *G. fusca*

The air dried root of *G. fusca* was extracted with ethyl acetate (7 L) and methanol (7 L) for 5 days by using Soxhlet extractor, respectively. Evaporation of the filtrate under reduced pressure (about 40 $^{\circ}$C) give ethyl acetate extract and methanol extract. The extraction procedure is shown in Scheme 1.

Isolation of compounds from the root of *G. fusca*

The ethyl acetate and methanol extract of the air dried root of *G. fusca* will be purified by using column chromatography techniques.



SCHEME 1 Extraction procedure of the air dried root of *G.fusca*

2. Isolation of compounds from the ethyl acetate extract of the root of *G. fusca*

The EtOAc extract (40.0 g) was fractionated by quick column chromatography (Merck silica gel 60 GF_{254}, 150 g), eluting with *n*-hexane-acetone and MeOH with increasing amounts of the more polar solvent. The eluates were examined by TLC and 14 combined fractions (Fr.1-14) were obtained.

2.1 Isolation of compound A (*β*-sitosterol, sss4192)

Fraction 3-5 (282.6 mg) were combined and chromatographed on a silica gel column (finer than 0.063 mm, 16 g) eluted with *n*-hexane and *n*-hexane-acetone (1% increment of acetone, each 100 mL). Eighteen fractions (7 mL per fraction) were collected and combined according to their TLC behavior to yield compound **A** (*β*-sitosterol, sss41952, 12.5 mg) as colorless solid (see Scheme 2).

2.2 Isolation of compounds B (cowanin, sss4099), C (cowaxanthone, sss4223), D (cowanol, sss4247) and F (*α*-mangostin, sss4384)

Fraction 7 (338.8 mg) to afford compound **B** (cowanin, sss4099, 93.6 mg) as yellow solid, and filtrated was rechromatographed over silica gel (finer than 0.063 mm, 60 g) with hexane-acetone as eluting solvent to give sixteen subfractions. Solid of subfraction 8 was prove to be compound **C** (cowaxanthone, sss4218, 48.8 mg) as yellow solid and filtrate from subfraction 9 (145.5 mg) was rechromatographed using *n*-hexane-acetone as eluents, with increasing amount of the more polar solvent to give seven subfractions (fr. 9.1-9.7). Fraction 9.7 was proved to be compound **F** (*α*-mangostin, 4384, 107.9 mg) as a yellow solid. Subfraction 12 (131.9 mg) was further subjected to silica gel chromatography employing solvent gradient hexane-acetone as eluting solvent to give compound **D** (cowanol, sss4382, 45.2 mg) as orange oil (see Scheme 2).

2.3 Isolation of compounds G (*β*-mangostin, sss4206)

From fraction 3-5 (282.6 mg) was chromatographed over silica gel (finer than 0.063 mm, 60 g) with *n*-hexane-acetone as eluting solvent to give eighteen subfractions. Subfraction 9 (906.0 mg) was rechromatographed using hexane-acetone as eluting solvent, with increasing amount of the more polar solvent to provide eight subfractions (fr. 9.1-9.9) Fraction 9.4 (168.5 mg) was further purified by sephadex LH-20, eluting with 100%MeOH, to afford compounds **G** (*β*-mangostin, sss4206, 65.2 mg) as yellow solid (see Scheme 2).

2.4 Isolation of compound D (cowanol, sss4343), I (isojacareubin, sss4246, 4434 and 4430), and E (fuscaxanthone G, sss4527)

Fraction 8 (2.22 g) was subjected to silica gel chromatography (finer than 0.063 mm, 50 g), eluting with CH_2Cl_2 and CH_2Cl_2-MeOH (1% increment of MeOH, each 200 mL), to give 16 subfractions as shown in Scheme 2.

Subfraction 9 (895.3 mg) gave compound **I** (isojacareubin, sss4246, 0.2 mg) as an orange solid and gave filtrate (83.6), it was rechromatographed on a silica gel column (18 g) eluted with hexane-acetone with increasing amounts of the more polar solvent to afford four fractions (fr. 9.1-9.4). Fraction 9.2 (66.6 mg) gave compound **D** (cowanol) as an orange solid and Fraction 9.4 (13.5 mg) gave an orange solid as **I**. Subfraction 12 (620.0 mg) was purified by Sephadex LH-20, using 100%MeOH as eluting solvent gave compound **E** (fuscaxanthone G, sss4527, 12.5 mg) as an orange solid and compound **D** (cowanol, sss 4528, 405.6 mg) as an orange solid.

2.5 Isolation of compound H (1,3,5,6,-tetrahydroxyxanthone sss4863)

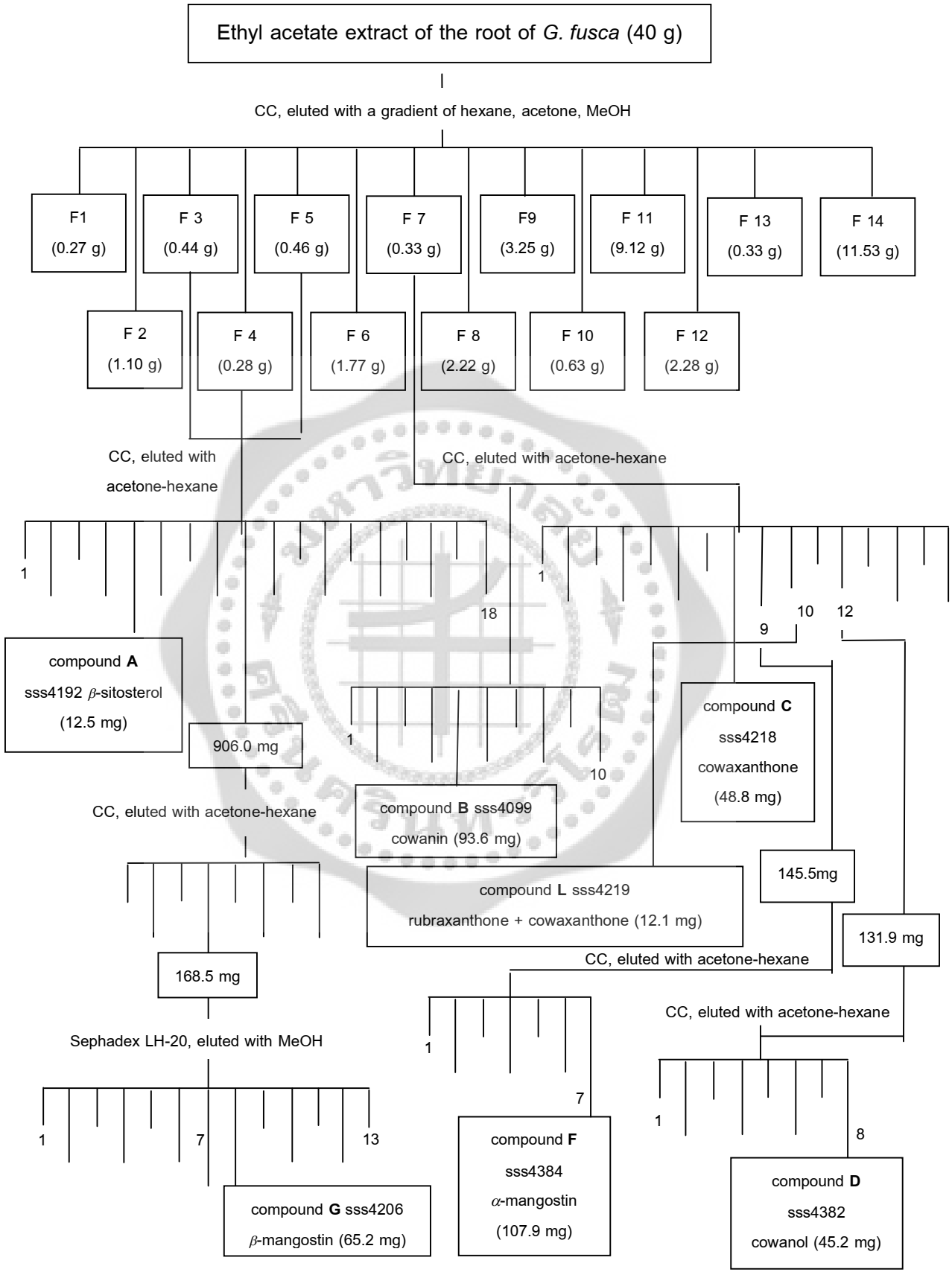
Fraction 10 (hexane–acetone, 6:4, 639.6 mg) was purified by CC, using CH_2Cl_2-EtOAc as eluting solvent, to give fifteen subfractions (10.1-10.15). Fraction 10.2 gave a biphenyl as red-brown solid. fraction 10.11 was purified by Sephadex LH-20, using 100%MeOH as eluting solvent gave compound **H** (1,3,5,6,-tetrahydroxyxanthone, sss 4863, 10.6 mg) as an pale yellow solid.

2.6 Isolation of compound J (morelloflavone, sss4665) and compound K (vokensiflavone, sss4757)

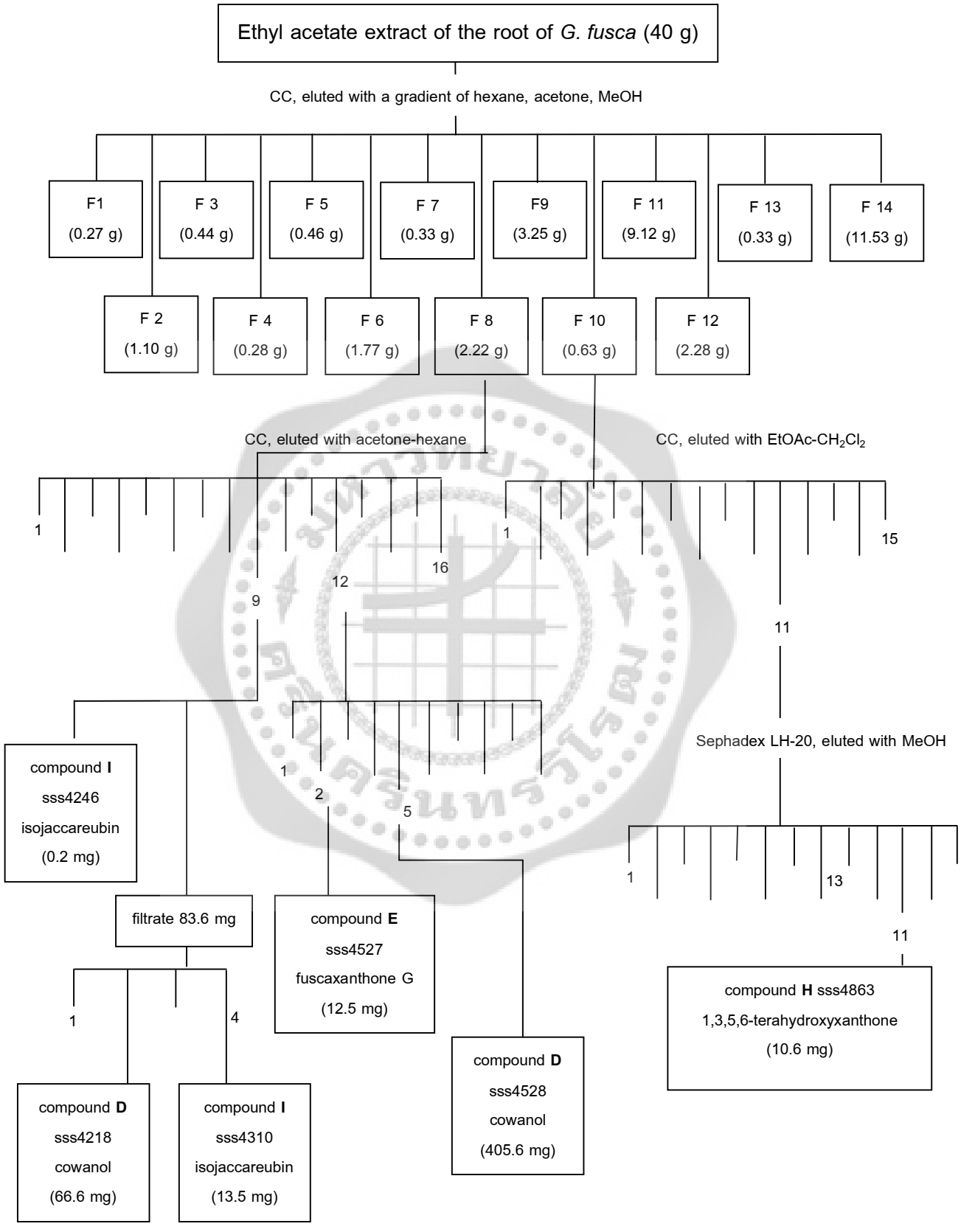
A portion of Fraction 11(500 mg, hexane - acetone, 5:5,) was purified by CC, using MeOH: H_2O: CH_2Cl_2 as eluting solvent to give compound **K** (vokensiflavone, sss4757, 15.8 mg) as a yellow solid. Another portion of Fraction 11 (500 mg) was purified by CC using hexane-ethyl acetate as eluting solvent to give 10 subfractions. A yellow solid of compound **J** (morelloflavone or fukugetin, sss4655, 129.2 mg) was isolated after repeated CC (using MeOH:H_2O:CH_2Cl_2 as eluting solvent) of subfraction 7 (160.6 mg).

2.7 Isolation of compound L (a mixture of rubraxanthone and cowaxanthone)

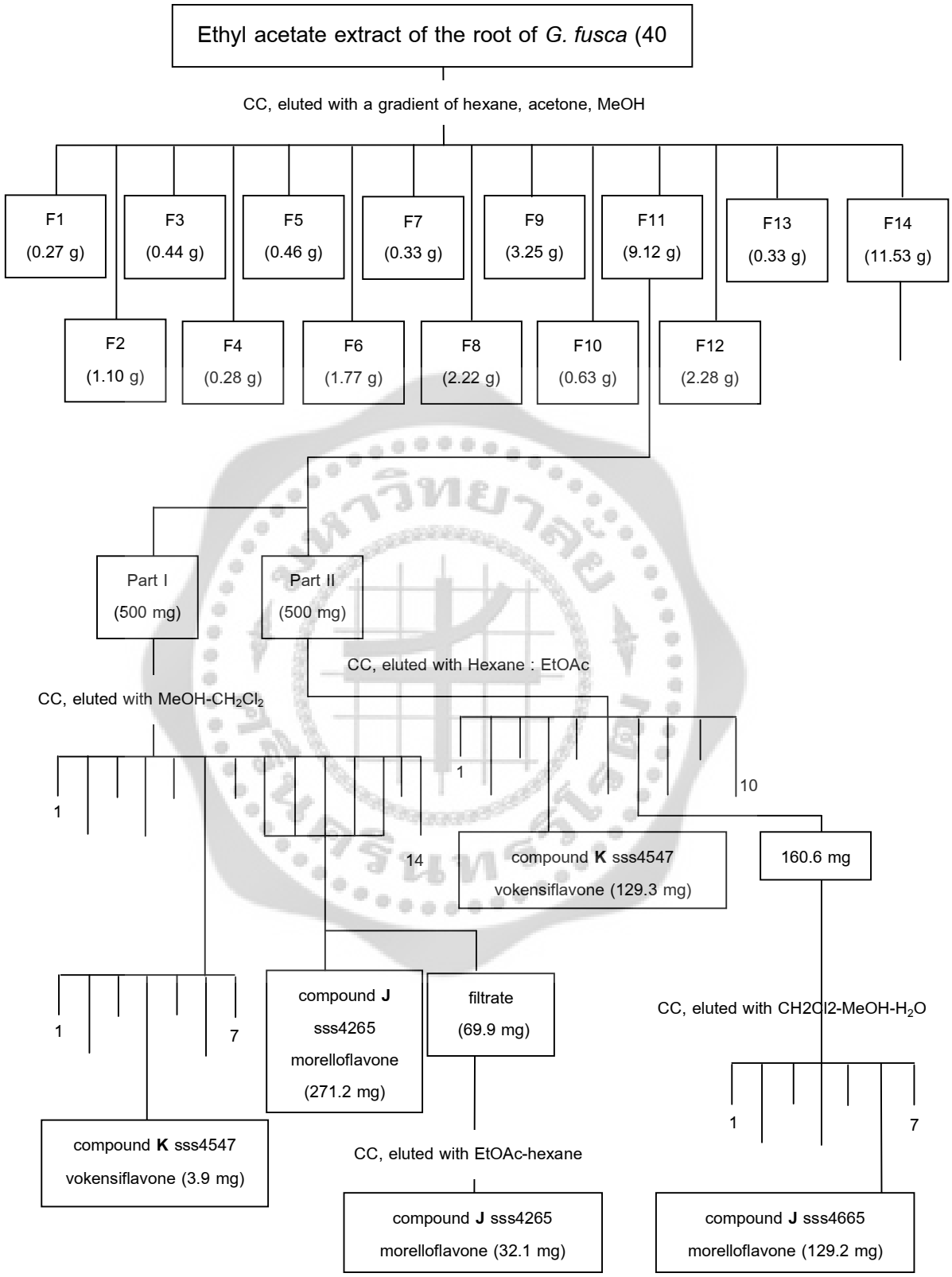
From fraction 7 (338.8 mg) was rechromatographed over silica gel (finer than 0.063 mm, 60 g), with hexane-acetone as eluting solvent to give sixteen subfractions. Subfraction 10 gave compound **L** (a mixture of xanthones, sss4219, 12.5 mg) as a yellow solid.



SCHEME 2 Isolation of EtOAc extract of the root of *G. fusca*



SCHEME 2 (continued) Isolation of EtOAc extract of the root of *G. fusca*



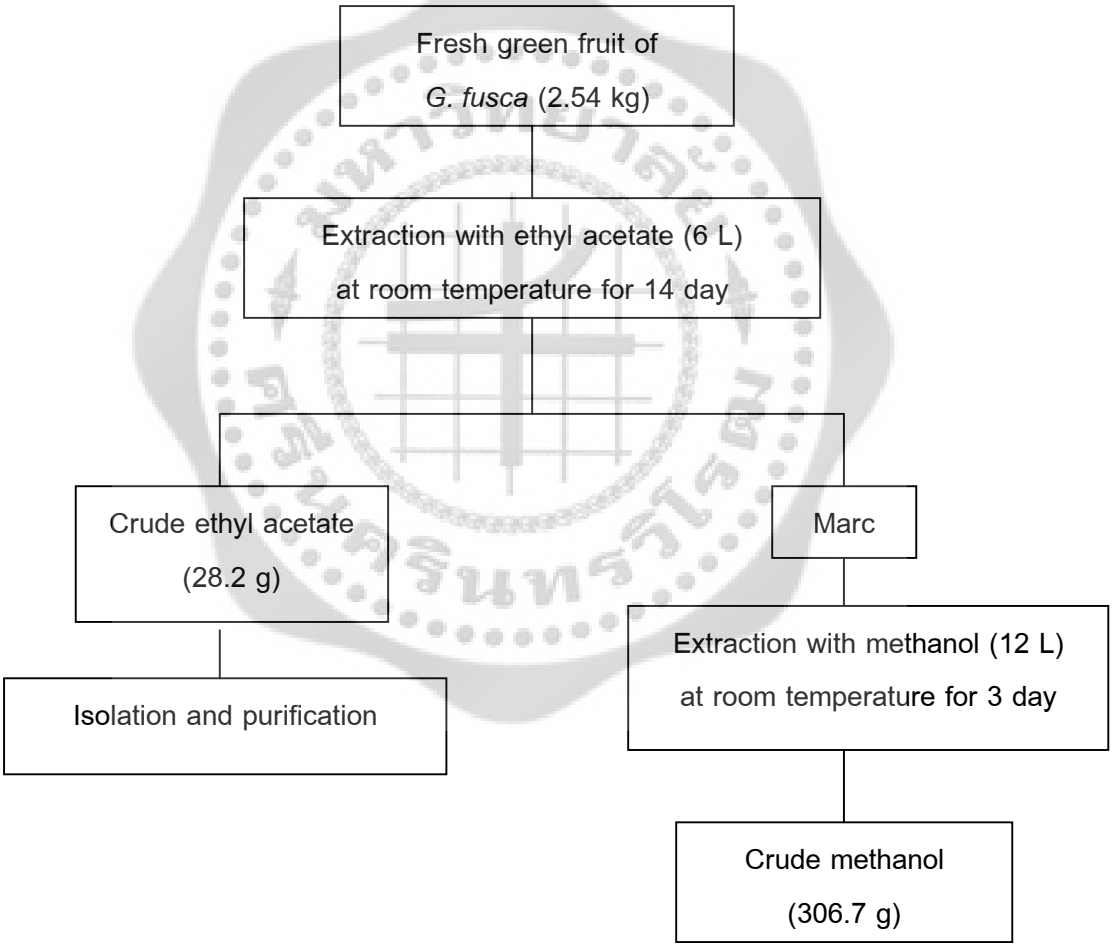
SCHEME 2 (continued) Isolation of EtOAc extract of the root of *G. fusca*

3. Extraction of the fresh green fruit of *G. fusca*

The fresh green fruit of *G. fusca* (2.54 kg) was extracted with 6 L ethyl acetate for 14 days at room temperature and 12 L methanol for 3 days at room temperature. Evaporation of the filtrate under reduced pressure (about 40 $^{\circ}$C) give ethyl acetate extract and methanol extract. The extraction procedure is shown in Scheme 3.

Isolation of compounds from the fresh green fruit of *G. fusca*

The ethyl acetate and methanol extract of the fresh green fruit of *G. fusca* will be purified by using column chromatographic techniques.



SCHEME 3 Extraction procedures of the fresh green fruit of *G. fusca*

4. Isolation of compounds from the ethyl acetate extract of the fresh green fruit of *G. fusca*

The EtOAc extract (14.6 g) was subjected to silica gel column chromatography (Merck silica gel 60 GF_{254}, 150 g), eluted with hexane-CH_2Cl_2, CH_2Cl_2-acetone with increasing amounts of the more polar solvent, successively to separate twelve main fractions. Successive treatment of each fraction with silica gel column and preparative TLC using appropriate combinations of solvent (hexane, CH_2Cl_2 and acetone) as eluting and developing solvents gave the following compounds.

4.1 Isolation of compound N (β-mangostin, sss4474) and R (fuscaxanthone A, sss3328)

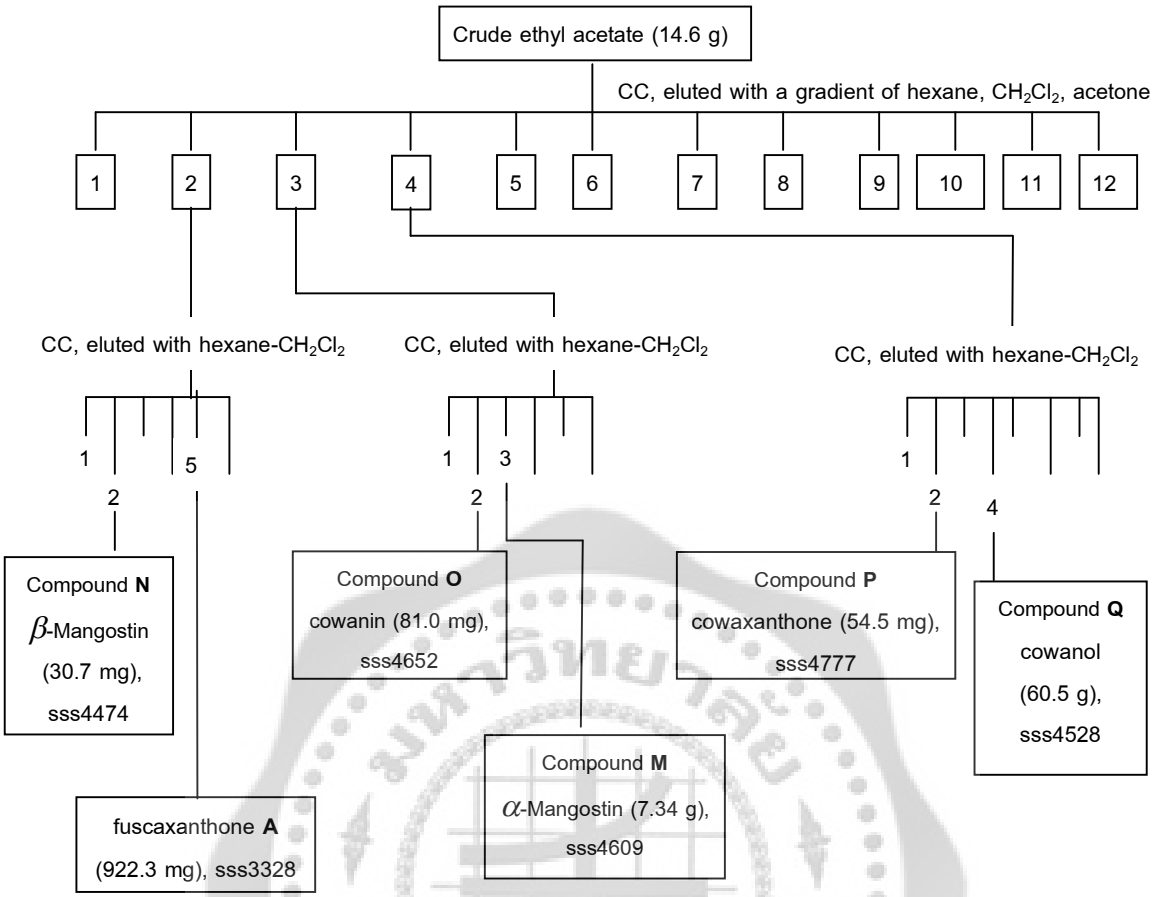
Fractions 2 was recolumn chromatographed (silica gel, using hexane-CH_2Cl_2 as eluting solvent) gave six subfractions. Subfraction 2 was to give compound **N** (β-mangostin, sss4474, 30.7 mg) as a yellow solid and subfractions 5 gave compound **R** (fuscaxanthone A, sss3328, 922.3 mg) as an orange oil.

4.2 Isolation of compound O (cowanin, sss4652) and M (α-mangostin, sss4609)

From fraction 3 was purified by column chromatography, using Hexane- CH_2Cl_2 as eluting solvent (1% increment of acetone, each 100 mL), to give six subfractions. Subfraction 2 gave compound **O** (cowanin, sss4652, 81.0 mg) as yellow solid and compound **M** (α-mangostin, sss4609, 7.34 g) as yellow solid was isolated from subfraction 3 (see Scheme 4).

4.3 Isolation of compound P (cowaxanthone, sss4777) and Q (cowanol, sss4528)

Fraction 4 (250.6 mg) was purified by column chromatography, using hexane-CH_2Cl_2 as eluting solvent (1% increment of acetone, each 100 mL), to give eight subfractions. An orange solid of compound **P** (cowaxanthone, sss4777, 54.5 mg) was isolated of subfraction 2. Subfraction 4 gave compound **Q** (cowanol, sss4528, 60.5 mg) as orange solid (see Scheme 4).



SCHEME 4 Isolation of EtOAc extract of fresh green fruit of *G. fusca*

Physical and spectral data of the isolated compounds from root of EtOAc extract

1. Compound A (β-sitosterol, sss4192)

Colorless solid 12.1 mg, soluble in CH_2Cl_2

R_f: 0.50 (20% acetone-hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1}: 3422, 2963, 2937, 1650, 1457, 1381, 1052, 1023, 970, 955, 800

UV λ_{max}^{MeOH} nm (log ε): 220.5(3.7)

^{1}H NMR: δ ppm, in $CDCl_3$; Table 5, Figure 5

2. Compound B (cowanin, sss4099)

Yellow solid 93.6 mg, soluble in EtOAc, acetone and MeOH

mp : 132-134 ^{o}C [lit. mp 135-137 ^{o}C (na Pattalung; et al. 1944: 365-366)

R_f: 0.50 (30% acetone-hexane, 2 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3429, 3289, 2965, 2914, 1642, 1608, 1580, 1455, 1374, 1278, 1186, 1161, 1076, 1049, 984, 901, 845, 591

UV λ_{max}^{MeOH} nm (log ε) : 353(4.0), 315(4.5), 256(4.6), 243(4.7)

^{1}H NMR : δ ppm, in $CDCl_3$; Table 6, Figure 6

3. Compound C (cowaxanthone, sss4223)

Yellow solid 48.8 mg, soluble in EtOAc, acetone and MeOH

mp : 192-196 ^{o}C [lit. mp 191-192 ^{o}C (na Pattalung; et al. 1944: 365-366)

R_f: 0.42 (30% acetone-hexane, 2 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3525 (sharp), 3123, 3085, 2913, 1634, 1613,1568, 1487,1443, 1309, 1288, 1225, 1190, 1161, 1094, 1016, 841, 768

UV λ_{max}^{MeOH} nm (log ε) : 361(4.5), 320(4.7), 257(4.8), 240(4.9)

^{1}H NMR : δ ppm, in $CDCl_3$ + MeOD; Table 7, Figure 7

4. Compound D (cowanol, sss4247)

Orange solid 547.4 mg, soluble in EtOAc, acetone and MeOH

mp : 120-124 °C [lit. mp 122-124 °C (na Pattalung; et al. 1944: 365-366)

R_f : 0.42 (30% acetone-hexane, 2 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3369 (broad), 2971, 2914, 2847, 1634, 1471, 1378, 1296, 1190, 1154, 1080, 1045, 986, 946, 838, 814

UV λ_{max}^{MeOH} nm (log ε) : 352(4.0), 314(4.5), 256(4.6), 243(4.7)

^{1}H NMR : δ ppm, in $CDCl_3$; Table 8, Figure 8

5. Compound E (fuscaxanthone G, sss4527)

Orange solid 12.5 mg, soluble in EtOAc, acetone and MeOH

mp : 250°C [140°C [lit, not reported, Ito; et al. 2003: 200-205]

R_f : 0.53 (30% acetone-hexane, 2 elutions), a pale green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3391(broad), 3220(broad), 2928, 1606, 1458, 1432, 1338, 1278, 1156, 1119, 1087, 1056, 838

UV λ_{max}^{MeOH} nm (log ε) : 342(4.3), 306(4.6), 244(4.9)

ESMS (+ve) *m/z* (% rel. intensity) : 479 $[M+H]^+$ (100) for $C_{29}H_{34}O_6$ + H

ESMS (-ve) *m/z* (% rel. intensity) : 477 $[M-H]^-$ (100) for $C_{29}H_{34}O_6$ - H

^{1}H NMR : δ ppm, in $CDCl_3$; Table 9, Figure 9

^{13}C NMR : δ ppm, in $CDCl_3$; Table 10, Figure 9

6. Compound F (α-mangostin, sss4384)

Yellow solid 107.9 mg, soluble in EtOAc, acetone and MeOH

mp : 178-180 °C [lit. mp mp 180-182 °C (Yates; & Stout. 1958: 1691-1700)]

R_f : 0.42 (30% acetone-hexane, 2 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3420, 3252, 2963, 2913, 1633, 1471, 1347, 1295, 1076, 1049, 1009, 985, 901, 848

UV λ_{max}^{MeOH} nm (log ε) : 353(4.1), 315(4.5), 256(4.6), 242(4.7)

^{1}H NMR : δ ppm, in $CDCl_3$+ MeOD; Table 11, Figure 12

7. Compound G (*β*-mangostin, sss4532)

Yellow solid 65.2 mg, soluble in CH_2Cl_2, EtOAc, acetone and MeOH

mp : 174-175 °C [lit. mp 176-180°C (Mahabusarakam & Wiriyachitra. 1987: 474-478)]

R_f : 0.43 (20% acetone-hexane, 4 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3398, 1646, 1602, 1570, 1483, 1456, 1425, 1381, 1282, 1203, 1170, 1147, 1111, 993, 840, 773

UV λ_{max}^{MeOH} nm (log ε) : 353(3.9), 314(4.5), 258(4.6), 243(4.6)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 12, Figure 13

8. Compound H (1,3,5,6-tetrahydroxyxanthone, sss4863)

Pale yellow solid 10.2 mg, soluble in in EtOAc, acetone and MeOH

mp : 138-140°C [lit, not reported, Farhm; & Chaudhuri. 1979: 2035-2038]

R_f : 0.25 (30% EtOAc-CH_2Cl_2, 2 elutions), a yellow coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3500, 3445, 3091(broad), 2956(broad), 2778(broad),, 2666, 2376, 1653, 1630, 1575, 1520, 1463, 1353, 1294, 1259, 1201, 1165, 1150, 1097, 1060, 810

UV λ_{max}^{MeOH} nm (log ε) : 323(4.6), 282(4.4), 249(5.0)

ESMS (-ve) *m/z* (% rel. intensity) : 259 $[M-H]^-$ (64), 519 $[2M-H]^-$ (100) for $C_{13}H_8O_6$ - H

^{1}H NMR : δ ppm, in $CDCl_3$ + DMSO-d_6; Table 13, Figure 14

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 13, Figure 14

9. Compound I (isojacareubin, sss4310)

Orange solid 13.5 mg, soluble in EtOAc, acetone and MeOH

mp : 168-172 °C [lit. 170-175 °C (Rath; et al. 1996: 513-520)]

R_f : 0.42 (30% acetone-hexane, 3 elutions), a blue coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{KBr} cm^{-1} : 3318, 1651, 1620, 1583, 1466, 1384, 1328, 1285, 1211, 1167. 1116

UV λ_{max}^{MeOH} nm (log ε) : 375(3.9), 329(4.2), 300(4.2), 256(4.8)

ESMS (-ve) *m/z* (% rel. intensity): 325 $[M-H]^-$ (22), 651 $[2M-H]^-$ (100)

^{1}H NMR: δ ppm, in acetone-d_6; Table 15, Figure 16

^{13}C NMR: δ ppm, in acetone-d_6; Table 15, Figure 16

10. Compound K (morelloflavone, sss4665)

Yellow solid 129.2 mg, soluble in acetone and MeOH

mp : 230-232 °C (d) [lit. (±) morelloflavone: 298-299 °C (d) and
(+) morelloflavone: 244-245 °C (d) (Konoshima; & Ikeshiro. 1969; 121-124) (+) morelloflavone: 280 °C (Li; et al. 2002: 8709-8717)

R_f : 0.42 (50% acetone-hexane), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{25.8}$: +161.6 ° (c = 0.20, MeOH) [lit. (±) morelloflavone: $[\alpha]_D^{29}$ = 0 (solvent not reported)
(+) morelloflavone: $[\alpha]_D^{29}$ = +170 °(MeOH)
(Konoshima; & Ikeshiro. 1969; 121-124)]
(+) morelloflavone: $[\alpha]_D^{25}$ = +188 °(c= 0.1, MeOH) (Li; et al. 2002: 8709-8717)

IR ν_{max}^{KBr} cm^{-1} : 3218, 2943, 2688, 1645, 1609, 1516, 1456, 1425, 1368, 1261, 1167, 1111, 1088, 1050, 1012, 967, 839 [lit :IR ν_{max}^{nujol} cm^{-1} : 3250(hydroxyl groups), 1645(conjugated γ-pyrone), 1600 and 1570 (benzene), (Konoshima; & Ikeshiro. 1969; 121-124)]

UV λ_{max}^{MeOH} nm (log Ɛ): 347(3.8), 287(4.0), 274(4.0), 254(3.8), 222(4.3)
[lit. 345 nm (4.13), 288(4.35), 275(4.33), 224(4.57, shoulder), (Konoshima; & Ikeshiro. 1969; 121-124)]

ESMS (-ve) *m/z* (% rel. intensity) : 555 $[M-H]^-$ (100) for $C_{30}H_{20}O_{11}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 17, Figure 19

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 17, Figure 19

11. Compound J (vokensiflavone, sss4547)

Yellow solid 19.6 mg, soluble in acetone and MeOH

mp : 220-221°C (d) [lit. (±) vokensiflavone: 290-293°C (d)
(Konoshima; Ikeshiro; & Miyahara. 1970: 4203-4206) and vokensiflavone: 244-245°C (Herbin; et al. 1970: 221)

R_f : 0.43 (10% MeOH : CH_2Cl_2), an orange coloration with anisaldehyde-H_2SO_4 reagent

$[\alpha]_D^{25.8}$: +142.0 (c = 0.10, MeOH) [lit., (±) vokensiflavone: $[\alpha]_D^{15}$ = 0 (c = 0.31, MeOH)
(Konoshima; & Ikeshiro. 1969; 121-124)]
(+) vokensiflavone-7-sulfate: $[\alpha]_D^{25}$ = +113
(c = 1.32, MeOH) (Li; et al. 2002: 8709-8717)

IR ν_{max}^{KBr} cm^{-1} : 3184, 2920, 2681, 1634, 1506, 1455, 1362, 1270, 1084, 969, 833839

[lit :IR ν_{max}^{nujol} cm^{-1} : 3100 (hydroxyl groups), 1640 and 1610 (conjugated γ-pyrone), 1570 and 1510 (benzene), (Konoshima; Ikeshiro; & Miyahara. 1970; 4203-4206]

UV λ_{max}^{MeOH} nm (log Ɛ) : 342(4.5), 325(4.6), 289(4.8), 221(5.0), 211(5.0) [lit. 330, 289, 275, 225(shoulder), (Konoshima; & Ikeshiro. 1969; 121-124)]

ESMS (+ve) *m/z* (% rel. intensity) : 539 $[M-H]^-$ (100) for $C_{30}H_{20}O_{10}$ - H

^{1}H NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 19, Figure 21

^{13}C NMR : δ ppm, in $CDCl_3$+ DMSO-d_6; Table 19, Figure 21

12. Compound L (a mixture of rubraxanthone and cowaxanthone)

Yellow solid 12.4 mg, soluble in EtOAc, acetone and MeOH

R_f : 0.42 (30% acetone-hexane, 2 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

^{1}H NMR : δ ppm, in $CDCl_3$; Table 21, Figure 23

Physical and spectral data of the isolated compounds from the fresh green fruit of EtOAc extract

1. Compound M (α-mangostin, sss4609)

Yellow solid 7.34 g, soluble in EtOAc, acetone and MeOH

UV λ_{max}^{MeOH} nm (log Ɛ) : 363(4.5), 342(4.7), 315(5.1), 256(5.2), 243(5.3)

^{1}H NMR : δ ppm, in $CDCl_3$; Figure 12

2. Compound N (β-mangostin, sss4474)

Yellow solid 30.7 mg, soluble in CH_2Cl_2, EtOAc, acetone and MeOH

UV λ_{max}^{MeOH} nm (log Ɛ) : 354(4.8), 314(5.3), 258(5.4), 243(5.5)

^{1}H NMR : δ ppm, in $CDCl_3$; Figure 13

3. Compound O (cowanin, sss4652)

Yellow solid 81.0 mg, soluble in EtOAc, acetone and MeOH

UV λ_{max}^{MeOH} nm (log ε) : 354(4.9), 315(5.4), 256(5.5), 244(5.6)

^{1}H NMR : δ ppm, in $CDCl_3$; Figure 6

4. Compound N (cowaxanthone, sss4777)

Yellow solid 54.5 mg, soluble in EtOAc, acetone and MeOH

UV λ_{max}^{MeOH} nm (log ε) : 361(5.1), 319(5.3), 258(5.5), 242(5.5)

^{1}H NMR : δ ppm, in $CDCl_3$; Figure 7

5. Compound P (cowanol, sss4528)

Orange solid 60.5 mg, soluble in EtOAc, acetone and MeOH

UV λ_{max}^{MeOH} nm (log ε) : 351(4.6), 316(5.1), 256(5.2), 243(5.3)

^{1}H NMR : δ ppm, in $CDCl_3$; Figure 8

6. Compound Q (fuscaxanthone A, sss3328)

Orange oil 922.3 mg, soluble in CH_2Cl_2, EtOAc, acetone and MeOH

R_f : 0.48 (20% acetone-hexane, 2 elutions), a green coloration with anisaldehyde-H_2SO_4 reagent

IR ν_{max}^{nujol} cm^{-1} : 3381, 2921, 2855, 1713, 1645, 1600, 1462, 1376, 1286, 1175, 1124, 1044, 987, 947, 889, 843

UV λ_{max}^{MeOH} nm (log ε) : 361(3.6), 328(4.1), 288(4.4), 239(4.1)

ESMS (+ve) *m/z* (% rel. intensity) : 476 $[M+H]^+$ (100) for $C_{29}H_{34}O_7$+ H

^{1}H NMR : δ ppm, in $CDCl_3$; Table 22, Figure 24

CHAPTER 4

RESULTS AND DISCUSSION

The ethyl acetate extract of the dried root of *Garcinia fusca* was investigated by column chromatographic methods to give one known triterpene (**A**), eight known xanthones (**B**-**I**), a mixture of xanthone (**L**) and two known biflavonoid (**J**-**K**) compounds (Table 3). The structures of these compounds were determined mainly based on their NMR data analysis, and by comparison with previously reported data.

TABLE 3 Compounds isolated from the root of *G. fusca*

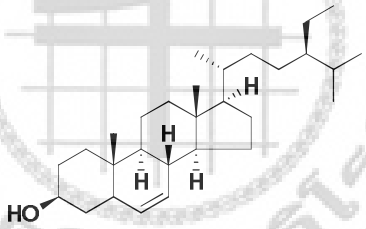
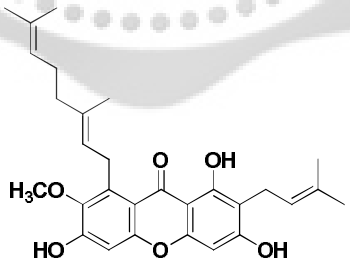
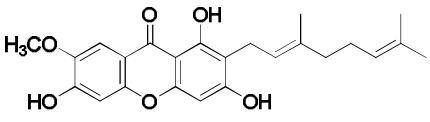
Compounds	Structure	Reference
A (β-sitosterol, sss4192, 12.5 mg)		Boonyaratavej & Petsom. 1991: 61-69
B (cowanin sss4099, 93.6 g)		Limnusont. 2007: 42-45
C (cowaxanthone sss4223, 48.8 mg)		Limnusont. 2007: 46-48. na Pattalung; et al. 1994: 365-368

TABLE 3 (continue)

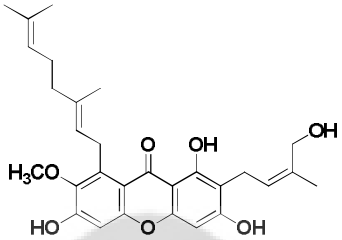
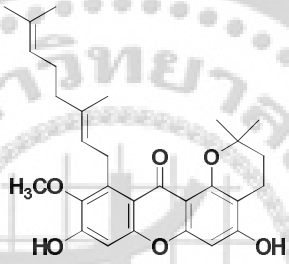
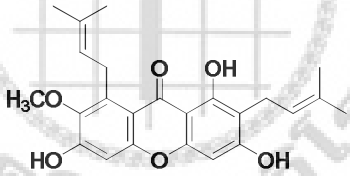
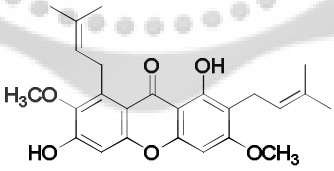
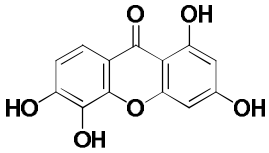
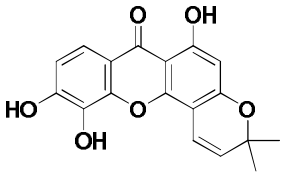
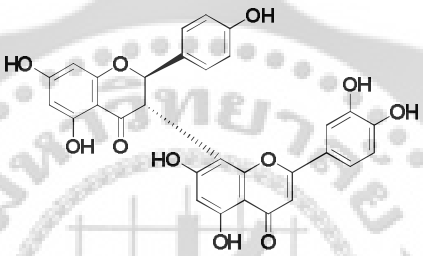
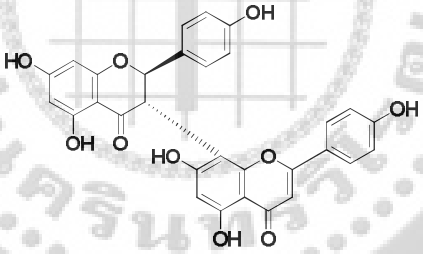
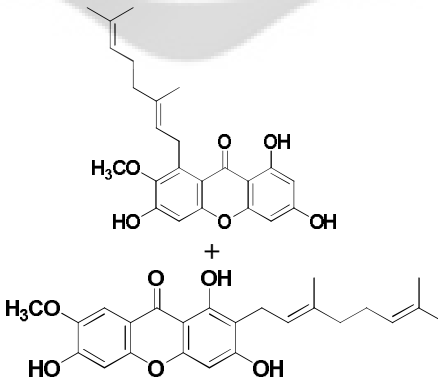
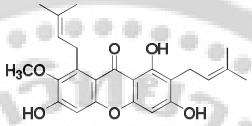
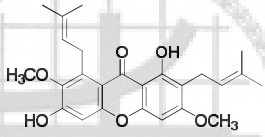
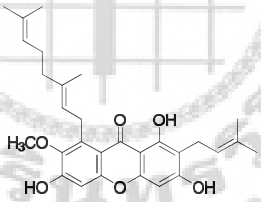
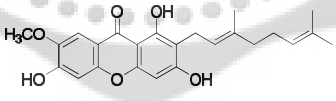
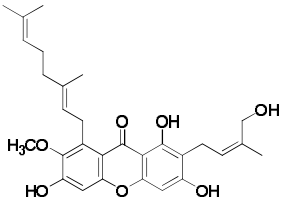
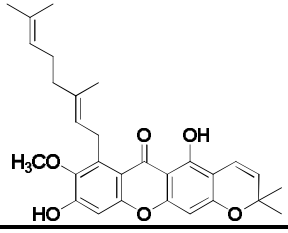
Compounds	Structure	Reference
D (cowanol sss4247, 547.4 mg)		Limnusont. 2007: 57-60. na Pattalung; et al. 1994: 365-368.
E (fuscaxanthone G sss4527, 12.5 mg)		Ito; et al. 2003: 200-205
F (α-mangostin sss4384, 107.9 mg)		Mahabusarakam; & Wiriyachitra. 1987: 474-478
G (β-mangostin sss4532, 65.2 mg)		Likhitwitayawuid; Phadungcharoen; & Krungkrai. 1998: 70-72
H (1,3,5,6,-tetrahydroxyxanthone sss4863, 10.2 mg)		Farhm; & Chaudhuri. 1979: 2035-2038

TABLE 3 (continue)

Compounds	Structure	Reference
I (isojacareubin sss4310, 13.5 mg)		Ishiguro; et al. 1993: 1583-1585
J (morelloflavone sss4665, 129.2 mg)		Terashima; et al. 2008: 407-413
K (vokensiflavone sss4668, 19.6 mg)		Chen; et al. 1957: 300-303
L (rubraxanthone+ cowaxanthone sss4868, 12.4 mg)		Limnusont. 2007: 49-52

The ethyl acetate extract of the fresh green fruit of *G. fusca* was investigated by chromatographic techniques to give six known xanthones (**M**-**R**). The structures of all compounds were elucidated by spectroscopic techniques, especially 1D and 2D NMR and MS including by comparison of their spectroscopic data with those reported in the literature.

TABLE 4 Compounds isolated from the fresh green fruit of *G. fusca*

Compounds	Structure	Reference
M (α-mangostin sss4609, 7.34 g)		Mahabusarakam; & Wiriyachitra. 1987: 474-478
N (β-mangostin sss4474, 30.7 mg)		Likhitwitayawuid; Phadungcharoen; & Krungkrai. 1998: 70-72
O (cowanin sss4652, 81.0 mg)		Limnusont. 2007: 42-45
P (cowaxanthone sss4777, 54.5 mg)		Limnusont. 2007: 46-48.
Q (cowanol sss4528, 60.5 mg)		Limnusont. 2007: 57-60. na Pattalung; et al. 1994: 365-368.
R (fuscaxanthone A sss3328, 922.3 mg)		Ito, et al. 2003: 200-205

1. Structure determination of compounds isolated from the ethyl acetate extract of *G. fusca*

1.1. Structure determination of compound **A** (β-sitosterol, sss4192)

Compound **A** was obtained as a colorless solid and its IR absorption bands exhibited for hydroxyl (3422 cm^{-1}), and olefinic double bond ($C=CH_2$) at 1650, 1457 cm^{-1}. The ^1H NMR spectra (Table 5, Figure 5) revealed a multiplet at δ_H 0.60-2.33 (*m*, C, CH, CH_2, and CH_3). From the NMR spectral pattern and chromatographic comparison with the authentic β-sitosterol in several solvent systems, the structure of compound **A** was identified as β-sitosterol (**21**).

β-sitosterol (**21**) is one of the phytosterols present in a large number of plants such as *Bridelia tomentosa* Bl., and its concentration is detected at low levels in the serum and tissues of healthy people eating fruits and vegetables (Pegel; et al. 1997: 263-268) and it was found in *Garcinia afzelii* ENGL. (Kamdem et al. 2006: 448-451). β-Sitosterol was reported to exhibit regulation of the proliferation and activities of peripheral blood lymphocytes and NK cells (Bouic; et al. 1996: 693-700), blood cholesterol level (Femandez et al. 2005: 57-70), and antipyretic activities (Gupta; et al. 1980: 157-163).

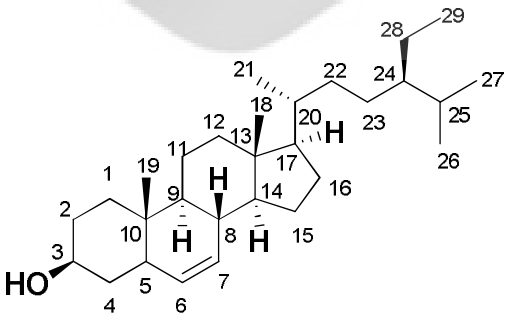


FIGURE 5 Structure of compound **A**

TABLE 5 Comparison of ^{1}H NMR data of compound **A** (sss4192) with β-sitosterol (Boonyaratavej; & Petsom. 1991: 61-69).

position	δ_H (*mult.*)	
	β-sitrosterol	compound **A**
	0.68-2.32 (*m*, C, CH, CH_2, CH_3)	0.60-2.33 (*m*, C, CH, CH_2, CH_3)
3	3.52 (*br*, OH)	3.49 (*br*, OH)
	5.09 (*t*, CH = CH)	5.09 (*t*, CH = CH)
6	5.35 (*d*, = CH)	5.33 (*d*, = CH)

1.2. Structure determination of compound **B** (cowanin, sss4099)

Compound **B** was the third major xanthone obtained as a yellow solid, and its IR spectrum of **B** showed the presence of a hydroxyl (3249 cm^{-1}), a conjugated carbonyl (1642 cm^{-1}) and aromatic moieties (1580 cm^{-1}). The UV spectrum displayed absorption bands at 243, 256, 315 and 353 nm which are the characteristic absorptions for xanthone skeletone. The ^{1}H NMR spectra (Table 6, Figure 6) obtained in $CDCl_3$ exhibited signal of a hydrogen bonded hydroxyl proton at δ 13.77 (*s*, 1-OH), a methoxy group at δ 3.77 (*s*, 7-OCH_3) and two aromatic protons at δ 6.18 (*s*, H-4) and δ 6.81 (*s,* H-5). A prenyl group was present, as was evident from the following resonances: one olefinic proton at δ 5.26 (*br t*, H-12), methylene protons at δ 3.43 (*d*, H-11) and two allylic methyl groups at δ 1.80 (*s*, H-14) and 1.75 (*s*, H-15). The remaining signals appeared as the typical signals of a geranyl unit. These signals were a doublet of methylene protons H-16 at δ 4.07, two broad triplets of the olefinic protons H-17 and H-21 at δ 5.26 and 5.00, respectively, two multiplets of the methylene protons H-19 and H-20 at δ 1.99 (4H) and three singlets of methyl groups H-23, H-24 and H-25 at δ 1.52, 1.82 and 1.57, respectively. From the NMR spectrum pattern and chromatographic comparison with the authentic cowanin in several solvent systems, the structure of compound **B** was identified as cowanin (**11**).

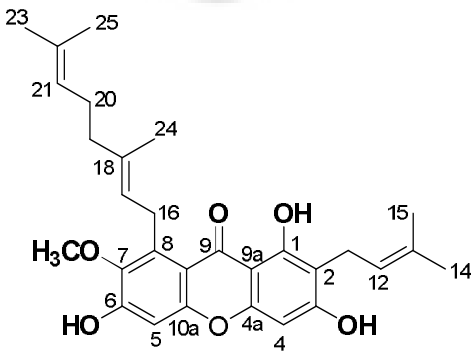


FIGURE 6 Structure of compound **B**

TABLE 6 Comparison of ^{1}H and ^{13}C NMR data of compound **B** (sss4099) with cowanin (**11**) (Limnusont. 2007: 42-45).

position	δ_H (*mult.*, *J* in Hz)		δ_C	
	cowanin	compound **B**	cowanin	compound **B**
1	13.77 (1H, *s*)	13.77 (1H, *s*)	160.1	160.5
2			108.5	108.4
3-OH	6.34 (1H, *br s*)	6.18 (1H, *br s*)	161.5	161.5
4	6.26 (1H, *s*)	6.27 (1H, *s*)	93.2	93.2
4a			154.2	154.4
5	6.79 (1H, *s*)	6.81 (1H, *s*)	101.5	101.5
6-OH	6.34 (1H, *br s*)	6.33 (1H, *br s*)	155.7	155.7
7			142.5	142.5
8			137.1	137.0
8a			112.2	112.1
9			181.9	181.9
9a			103.6	103.5
10a			155.0	155.0
11	3.42 (2H, *d*, *J* = 6.7)	3.43 (2H, *d*, *J* = 6.7)	21.4	21.4
12	5.25 (1H, *br t*, *J* = 6.7)	5.26 (1H, *br t*, *J* = 6.7)	121.4	121.4
13			131.2	131.2
14, 15	1.74 (3H, *s*)	1.75 (3H, *s*)	25.8	25.8
	1.80 (3H, *s*)	1.80 (3H, *s*)	17.8	17.8
16	4.06 (2H, *d*, *J* = 6.7)	4.07 (2H, *d*, *J* = 6.7)	26.4	26.5
17	5.25 (2H, *br t*, *J* = 6.7)	5.26 (2H, *br t*, *J* = 6.7)	123.2	123.1
18			135.5	135.6
19, 20	1.99 (4H, *m*)	1.99 (4H, *m*)	39.6	39.6
			26.5	26.5
21	5.00 (1H, *br t*, *J* = 6.0)	5.00 (1H, *br t*, *J* = 6.0)	124.2	124.2
22			132.1	135.5
23	1.52 (3H, *s*)	1.52 (3H, *s*)	17.6	17.6
24	1.82 (3H, *s*)	1.82 (3H, *s*)	16.4	16.4
25	1.57 (3H, *s*)	1.57 (3H, *s*)	25.5	25.6
7-OCH_3	3.77 (3H, *s*)	3.77 (3H, *s*)	62.0	62.0

1.3. Structure determination of compound **C** (cowaxanthone, sss4223)

Compound **C** was obtained as a yellow solid, and its UV spectrum exhibited absorption bands at 240, 257, 320 and 361 nm which are the characteristic absorptions for xanthone skeletone. Compound **C** had absorption bands of hydroxyl groups (3525 cm^{-1}), and a conjugated carbonyl group (1634 cm^{-1}) in its IR spectrum. The ^{1}H NMR spectra (Table 7, Figure 7) exhibited signals of chelated phenolic hydroxyl proton at δ 13.27 (*s*, 1-OH), one methoxy group at δ 3.96 (*s*, 7-OCH_3) and three aromatic protons at δ 6.33 (*s*, H-4), 6.87 (s, H-5) and 7.55. The aromatic proton at δ 7.55 was assigned to be H-8 as it was deshielded by the C-9 carbonyl group. A geranyl group was also evident: three methyl singlets at δ 1.64, 1.93 and 1.79, a doublet (*J* = 6.9 Hz) for methylene protons at δ 3.40 and an olefinic proton signal at δ 5.25. From the NMR spectrum pattern and chromatographic comparison with the authentic cowaxanthone in several solvent systems, the structure of compound **C** was identified as cowaxanthone (**9**).

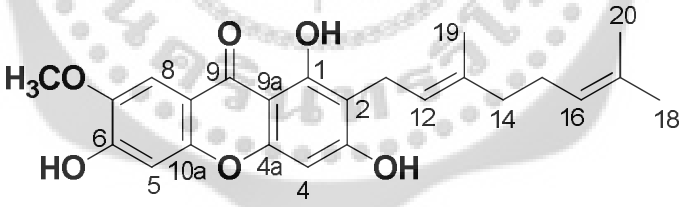


FIGURE 7 Structure of compound **C**

TABLE 7 Comparison of ^{1}H NMR data of compound **C** (sss4223) with cowaxanthone (**9**) (Limnusont. 2007: 46-48.) in acetone-d_6 and (na Pattalung; et al. 1994: 365-368.) in $CDCl_3$

position	δ_H (*mult.*, *J* in Hz)		
	cowaxanthone (acetone-d_6)	cowaxanthone ($CDCl_3$)	compound **C** ($CDCl_3$ + MeOH)
1	13.41 (1H, *s*)	13.45 (*s*)	13.27 (1H, *s*)
3-OH	-	-	-
4	6.46 (1H, *s*)	6.37 (*s*)	6.33 (1H, *s*)
5	6.89 (1H, *s*)	6.91 (*s*)	6.87 (1H, *s*)
6-OH	-	-	-
8	7.53 (1H, *s*)	7.58 (*s*)	7.55 (1H, *s*)
11	3.35 (2H, *d*, *J* = 7.0)	3.41 (br *d*, *J* =7.0)	3.40 (2H, *d*, *J* = 6.9)
12	5.30 (1H, *br t*, *J* = 7.2)	5.30 (*br t*, *J* = 7.0)	5.25 (1H, *br t*, *J* = 6.9)
14, 15	1.95 (4H, *m*)	2.06 (*m*)	1.98 (3H, *s*)
	1.95 (4H, *m*)	2.06 (*m*)	1.98 (3H, *s*)
16	5.06 (1H, *br t*, *J* = 7.2)	5.06 (*br t*, *J* = 7.0)	5.03 (1H, *br t*, *J* = 6.9)
18	1.58 (3H, *s*)	1.58 (s)	1.64 (3H, s)
19, 20	1.78 (3H, *s*)	1.83 (s)	1.93 (3H, s)
	1.53 (3H, *s*)	1.66 (*s*)	1.79 (3H, *s*)
7-OCH_3	3.96 (3H, *s*)	4.00 (*s*)	3.96 (3H, *s*)

1.4. Structure determination of compound **D** (cowanol, sss4247)

Compound **D** was the major xanthone obtained as an orange solid, and was more polar than compound **B** (R_f value of 0.42). In the IR spectrum, characteristic absorptions of a xanthone were observed at 3369 cm^{-1} (OH), 1634 cm^{-1} (conjugated carbonyl) and 1471 cm^{-1} (aromatic moieties). The UV spectrum exhibited absorption bands at 243, 256, 314 and 352 nm, which are the characteristic absorptions for xanthone skeleton. The ^{1}H NMR spectra (Table 8, Figure 8) showed the presence of a chelated phenolic hydroxyl group at δ 13.82, a singlet resonance of methoxy groups at δ 3.77 (7-OCH_3) and two singlet signals of two isolated aromatic protons H-4 and H-5 at δ 6.28 and 6.80, respectively. Two side chains were detected: a geranyl side chain and a prenyl unit with a hydroxyl group. The signals of the geranyl unit appeared as follows: two olefinic protons at δ 5.24 (H-17) and 5.00 (H-21), three sets of methylene groups at δ 4.07 (H-16), 2.02 (H-19 and H-20) and three singlets vinylic methyl groups at δ 1.76 (H-24), 1.58 (H-23) and 1.52 (H-25). Other signals were assigned to a 4-hydroxy-3-methyl-2-butenyl group, these, a broad triplet of olefinic proton at δ 5.44 (H-12), a doublet of benzylic methylene protons at δ 3.50 (H-11), a singlet of two oxymethylene protons at δ 4.33 (H-14) and a singlet of vinylic methyl proton at δ 1.80 (H-15). From the NMR spectrum pattern and chromatographic comparison with the authentic cowaxnol in several solvent systems, thus compound **D** was elucidated as cowanol (**14**).

Cowaxanthone (**9**), cowanin (**11**) and cowanol (**14**) was found in *Garcinia* plants such as *G. cowa* (na Pattalung; et al. 1994, 365-366), *G. fusca* (Ito; et al. 2003, 200-205) and *G. oliver* (Ha; et al. 2009: 830-834). It was reported to exhibit significant antibacterial activities (Panthong; et al. 2006: 999-1004), tumor-promoting inhibition (Ito; et al. 2003: 200-205), radical scavenging activity (Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153) and cytotoxic activities (Ha; et al. 2009: 830-834).

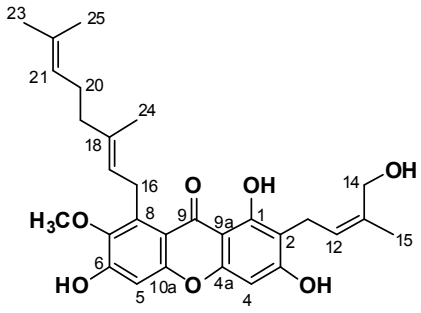


FIGURE 8 Structure of compound **D**

TABLE 8 Comparison of ^{1}H NMR data of compound **D** (sss4247) with cowanol (**14**)

position	δ_H (*mult.*, *J* in Hz)		
	[a]cowanol	[b]cowanol	compound **D**
1	13.83 (1H, *s*)	13.96 (*s*)	13.82 (1H, *s*)
4	6.27 (1H, *s*)	6.30 (*s*)	6.28 (1H, *s*)
4a			
5	6.78(1H, *s*)	6.80 (*s*)	6.80 (1H, *s*)
11	3.48 (2H, *br d*, *J* = 7.7)	3.48 (*br d*, *J* = 7.7)	3.50 (2H, *d*, *J* = 7.8)
12	5.44 (1H, *br t*, *J* = 7.5)	5.47 (*br t*, *J* = 7.0)	5.44 (1H, *br t*, *J* = 7.8)
14-OH	4.33 (2H, *br s*)	4.35 (*s*)	4.33 (2H, *s*)
15	1.76 (3H, *s*)	1.82 (*s*)	1.80 (3H, *s*)
16	4.06 (2H, *br d*, *J* = 5.5)	4.09 (*d*, *J* = 7.0)	4.07 (2H, *d*, *J* = 6.0)
17	5.24 (1H, *br t, J* = 7.5)	5.24 (*br t*, *J* = 7.0)	5.24 (1H, *br t*, *J* = 6.0)
18			
19, 20	1.99 (4H, *m*)	2.03 (*m*)	2.02 (4H, *m*)
	1.99 (4H, *m*)	2.03 (*m*)	
21	5.00 (1H, *br t*, *J* = 7.5)	5.00 (*br t*, *J* = 7.0)	5.00 (1H, *br t*, *J* = 6.0)
23	1.57 (3H, *s*)	1.59 (*s*)	1.58 (3H, *s*)
24	1.80 (3H, *s*)	1.79 (*s*)	1.76 (3H, *s*)
25	1.52 (3H, *s*)	1.54 (*s*)	1.52 (3H, *s*)
7-OCH_3	3.77 (3H, *s*)	3.80 (*s*)	3.77 (3H, *s*)

[a]Limnusont. 2007: 57-60, $CDCl_3$

[b]na Pattalung; et al. 1994: 365-368, $CDCl_3$

1.5. Structure determination of compound **E** (fuscaxanthone G, sss4527)

Compound **E** was obtained in a small amount as an orange solid. The peak at m/z 479 in its ESMS data was compatible with the molecular formula $C_{29}H_{34}O_6$. The IR spectrum exhibited absorption bands for hydroxyl (3391 cm^{-1}), chelated carbonyl (1606 cm^{-1}), and aromatic ring (1458 cm^{-1}) whilst the UV spectrum revealed four maxima at 244, 306 and 342 nm, which are the characteristic absorptions for xanthone skeletone. The ^{1}H NMR spectrum (Table 9, Figure 9) was shown to be quite similar to that of compound **B** (cowanin), except for the appearance of 2,2-dimethyldihydropyran ring [δ 2.61 (*t*, *J* = 5.9 Hz, H-11); δ 1.57 (*br t*, *J* = 5.9 Hz, H-12); δ 1.37 (*s*, H-14 and H-15)] and the lack of a typical lower field hydrogen-bonded OH signal. Thus the presence of thes 2,2-dimethyldihydropyran ring showed be located at C-2 and C-1 (Table 10, Figure 10). The geranyl group was located at C-8 according to correlations of benzylic methylene protons H-16 to C-7 and the deshielding effect of the C-9 carbonyl group on H-16. In addition the methoxy protons at δ 3.75 enhanced the signal of H-16 in its NOESY spectrum (Table 10, Figure 11), thus the methyl group should be at C-7. On the basis of these data coupled with other HMBC correlations, we therefore propose the structure of **E** for fuscaxanthone G. Fuscaxanthone G (**7**) was found in *G. fusca* and it was reported to exhibit significant tumor-promoting inhibition (Ito; et al. 2003: 200-205).

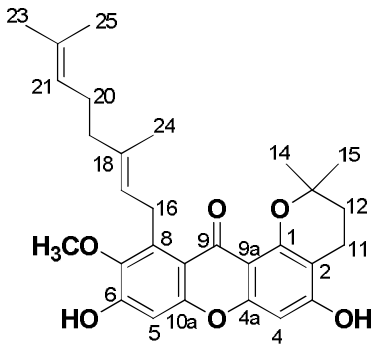


FIGURE 9 Structure of compound E

TABLE 9 Comparison of ^{1}H NMR data of compound **E** (sss4527) with fuscaxanthone G (**7**) (Ito; et al. 2003: 200-205).

position	δ_H (*mult.*, *J* in Hz)	
	fuscaxanthone G (**7**)	compound **E**
4	6.54 (1H, *s*)	6.29 (1H, *s*)
5	6.77 (1H, *s*)	6.65 (1H, *s*)
11	2.65 (2H, *t*, *J* = 6.6)	2.61 (2H, *t*, *J* = 5.9)
12	1.79 (2H, *t*, *J* = 6.6)	ca. 1.75 (*overlapping signal*)
14	1.40 (3H, *s*)	1.37 (3H, *s*)
15	1.40 (3H, *s*)	1.37 (3H, *s*)
16	4.07 (2H, *d*, *J* = 6.6)	4.07 (2H, *d*, *J* = 5.7)
17	5.38 (*m*)	5.32 (*m*)
19	1.94 (2H, *m*)	1.92 (2H, *m*)
20	2.01 (2H, *m*)	1.98 (2H, *m*)
21	4.99 (*m*)	4.97 (*m*)
23	1.51 (3H, *s*)	1.54 (3H, *s*)
24	1.57 (3H, *s*)	1.58 (3H, *s*)
25	1.79 (3H, *s*)	1.75 (3H, *s*)
7-OCH_3	3.79 (3H, *s*)	3.75 (3H, *s*)

Signals without multiplicity were assigned from COSY

TABLE 10 ^{1}H, ^{13}C NMR and 2D NMR data of compound **E** (sss4527).

position	δ_H (*mult.*, *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1		156.9		
2		107.5		
3		156.5		
4	6.29 (1H, *s*)	93.6		
4a		154.4		
5	6.65 (1H, *s*)	100.8		
6		153.1		
7		142.4		
8		136.9		
8a		115.0		
9		181.9		
9a		104.3		
10a		158.6		
11	2.61 (2H, *t*, *J* = 5.9)	16.9		
12	1.75 (2H, *br t*, *J* = 5.9)	31.3		
13		75.5		
14	1.37 (3H, *s*)	26.4	C-12, C-13, C-15	
15	1.37 (3H, *s*)	26.4	C-12, C-13, C-14	
16	4.07 (2H, *d*, *J* = 5.7)	26.3	C-7	H-17, H-21, H-24
17	5.32 (*m*)	123.9		7-OCH_3, H-16
18		134.7		
19	1.92 (2H, *m*)	39.6	C-20	
20	1.98 (2H, *m*)	26.6		
21	4.97 (*m*)	124.4		7-OCH_3, H-16, H-23, H-25, H-20,
22		131.0		
23	1.54 (3H, *s*)	25.5	C-21, C-22, C-25	
24	1.58 (3H, *s*)	16.4	C-17, C-18, C-19, C-20	
25	1.75 (3H, *s*)	17.6	C-21, C-22, C-23	
7-OCH_3	3.75 (3H, *s*)	67.7	C-7	H-17, H-21, H-19, H-24,

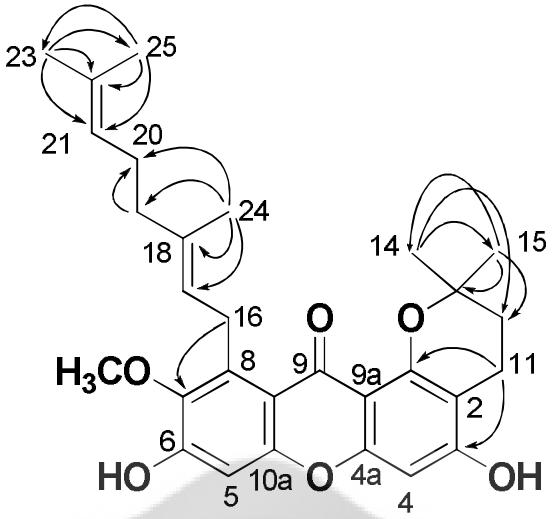


FIGURE 10 Selected HMBC correlations for compound **E**

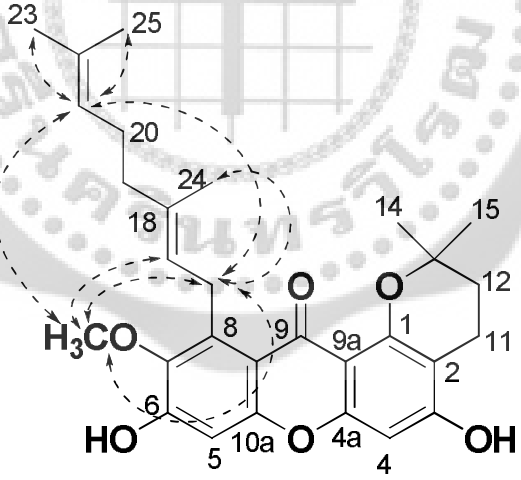


FIGURE 11 Selected NOESY correlations for compound **E**

1.6. Structure determination of compound **F** (α-mangostin, sss4384)

Compound **F** was the second major xanthone obtained as a yellow solid. The UV spectrum (242, 256, 315 and 353 nm) and the IR exhibited absorption bands at ν_{max}^{KBr} 3420 (OH), 1633 (chelated C=O), 1471 (aromatic ring) cm^{-1} revealed that compound **F** was also a xanthone. The ^{1}H NMR spectra (Table 11, Figure 12) showed the presence of a chelated phenolic hydroxyl group at δ 13.68 and one methoxy group at δ 3.75 (7-OCH_3), two singlet aromatic protons H-4 at δ 6.22 and H-5 at δ 6.73, and showed the presence of two prenyl groups (δ 5.23, 2H, *br t*; δ 4.05, 2H, *d*, *J* = 6.0 Hz; δ 3.36, 2H, *d*. *J* = 6.9 Hz; δ 1.79, 2 x CH_3; δ 1.69, 1.50, 2 x CH_3). From the NMR spectrum pattern and chromatographic comparison with the authentic α-mangostin in several solvent systems, suggesting that compound **F** was a α-mangostin (**13**).

α-Mangostin (**13**) was found as the major xanthone *G. mangostana* (Yates; & Stout. 1958: 1691-1699) and also found in orther *Garcinia* plants such as *G. cowa* (Panthong; et al; 2006: 999-1004) and *G. fusca* (Ito; et al. 2003: 200-205).

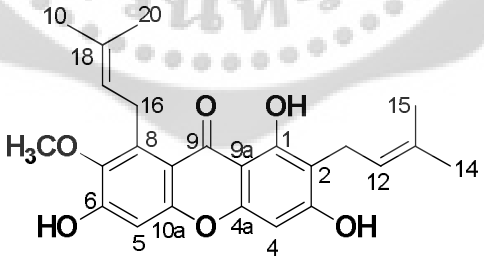


FIGURE 12 Structure of compound **F**

TABLE 11 Comparison of ^{1}H NMR data of compound **F** (sss4532) with α-mangostin (**13**) (Mahabusarakam; & Wiriyachitra. 1987: 474-478).

position	δ_H (*mult.*, *J* in Hz)	
	α-mangostin[a]	compound **F**[b]
1	13.80 (1H, *s*)	13.68 (1H, *s*)
3-OH	6.61 (1H, *s*)	-
4	6.28 (1H, *s*)	6.22 (1H, *s*)
5	6.82 (1H, *s*)	6.73 (1H, *s*)
6-OH	6.31 (1H, *s*)	-
11	3.45 (2H, *d*, *J* = 7.0)	3.36 (2H, *d*, *J* = 6.9)
12	5.28 (1H, *br t*, *J* = 7.0)	5.24 (1H, *br t*, *J* = 6.9)
14, 15	1.70 (3H, *s*)	1.69 (3H, *s*)
	1.78 (3H, *s*)	1.50 (3H, *s*)
16	4.11 (2H, *d*, *J* = 7.0)	4.05 (2H, *d*, *J* = 6.0)
17	5.28 (1H, *d*, *J* = 7.0)	5.22 (1H, *br t*, *J* = 6.0)
19, 20	1.82 (3H, *s*)	1.79 (3H, *s*)
	1.85 (3H, *s*)	1.79 (3H, *s*)
7-OCH_3	3.81 (3H, *s*)	3.75(3H, *s*)

[a] in $CDCl_3$

[b] in $CDCl_3$ + MeOD

1.7. Structure determination of compound **G** (β-mangostin, sss4532)

Compound **G** was obtained as a yellow solid, and its IR spectrum of G showed the presence of a hydroxyl (3398 cm^{-1}), a conjugated carbonyl (1646 cm^{-1}) and aromatic moieties (1602 cm^{-1}). The UV spectrum displayed absorption bamds at 243, 258, 314 and 353 nm revealed that compound **G** was also a xanthone. The ^{1}H NMR spectra (Table 12, Figure 13) indicated the presence of a xanthone skeleton as in compound G. The ^{1}H NMR spectra of compound G was similar to those of compound **F** (α-mangostin) but compound G had an additional methoxy group at δ 3.88 was assigned at C-3. From the ^{1}H NMR spectrum pattern and chromatographic comparison with the authentic β-mangostin in several solvent systems, the structure of compound **G** was identified as β-mangostin (**10**).

β-Mangostin (**10**) was found in *Garcinia* plants such as *G. mangostana* (Yates; & Stout. 1958: 1691-1699) *G. cowa* (Panthong; et al; 2006: 999-1004) and *G. fusca* (Ito; et al. 2003: 200-205) and *G. oliver* (Ha; et al. 2009: 830-834).

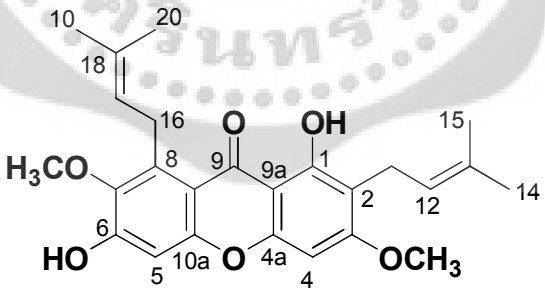


FIGURE 13 Structure of compound **G**

TABLE 12 Comparison of ^{1}H NMR data of compound **G** (sss4192) with β-mangostin (**10**) (Likhitwitayawuid; Phadungcharoen; & Krungkrai; 1998: 70-72).

position	δ_H (*mult.*, *J* in Hz)	
	β-mangostin	compound **G**
1	13.39 (1H, *s*)	13.39 (1H, *s*)
3-OCH_3	3.88 (3H, *s*)	3.88 (3H, *s*)
4	6.32 (1H, *s*)	6.32 (1H, *s*)
5	6.81 (1H, *s*)	6.81 (1H, *s*)
6-OH	6.30 (1H, *s*)	-
11	3.33 (2H, *d*, *J* = 7.0)	3.33 (2H, *d*, *J* = 7.2)
12	5.21 (1H, *br t*, *J* = 7.0)	5.21 (1H, *br t*, *J* = 7.2)
14, 15	1.78 (3H, *s*)	1.55 (3H, *s*)
	1.67 (3H, *s*)	1.66 (3H, *s*)
16	4.08 (2H, *br t*, *J* = 6.4)	4.07 (2H, *d*, *J* = 6.3)
17	5.24 (1H, *br t*, *J* = 6.4)	5.21 (1H, *br t*, *J* = 6.3)
19, 20	1.81 (3H, *s*)	1.77 (3H, *s*)
	1.67 (3H, *s*)	1.81(3H, *s*)
7-OCH_3	3.79(3H, *s*)	3.79(3H, *s*)

1.8. Structure determination of compound **H** (1,3,5,6-tetrahydroxyxanthone, sss4863)

Compound **H** was obtained as a pale pale yellow solid, m.p 138-140^{O}C, with the R_f value of 0.25 (30% EtOAc-CH_2Cl_2, 2 elutions). Its IR spectrum exhibited absorption bands for hydroxyl (3500 cm^{-1}), conjugated carbonyl (1653 cm^{-1}) and aromatic ring (1630 and 1575 cm^{-1}) and its UV spectrum displayed absorption bamds at 323, 282 and 249 nm, which are the characteristic absorptions for xanthone skeletone. The ^{1}H NMR spectrum (Table 13, Figure 14) was revealed an chelated hydroxyl (δ_H 12.71 (*s*), two aromatic *ortho* coupled protons at δ_H 6.49 and 7.14 (2*d, J* = 8.7 Hz, H-7, H-8) and two aromatic *meta* coupled protons at δ_H 5.79 and 6.04 (2*d, J* = 2.0 Hz, H-2, H-3). The molecular ion at m/z 259 in the ESMS and the ^{13}C NMR data had shown the molecular formula to be $C_{13}H_8O_6$.

Connections among these subsgroups were provided by analysis of its HMBC and NOESY spectra (Table 14; Figures 15). The HMBC correlations were observed for chelated hydroxyl proton at δ_H 12.71. (1-OH) to C-1 (δ_C 162.4), C-2 (δ_C 97.2) C-9a (δ_C 101.2) and carbonyl C-9 (δ_C 179.3), together with correlations of H-2 (δ_H 6.91) to C-1, C-3 and C-4 and C-9a and H-4 to C-2, C-3, C-4a and C-9a indicating that two isolated aromatic proton was attached to the ring B unit. HMBC correlations from H-7 and H-8 to C-5, C-6, C-7, C-9 (δ_C 181.5) and C-10a confirmed the connection between two aromatic *ortho* coupled protons was attached to the ring A of xanthone unit. Compound **H** was thus characterized as 1,3,5,6-tetrahydroxyxanthone. In addition the ^{13}C NMR data of **H** was similar to that of 1,3,5,6-tetrahydroxyxanthone. This is the first time to report ^{1}H NMR data of H.

1,3,5,6-Tetrahydroxyxanthone was isolated before from *Hypericum* plants such as *H. androsaemum* (Nieslen; et al. 1979: 301-304) and *H. patulum* (Ishiguro; et al. 1993: 1583-1585), and was found in *Calophyllum brasiliens*e (King; & manning. 1953: 3932-3937), *C. sclerophyllum* (Jackon; Locksley; & Scheinmann. 1966: 178-181), *Canscora decussata* (Ghosal; & Chaudhuri. 1975: 888-889) and *Cratoxylum cochinchinense* (Sai; et al. 1995: 1521-1528).

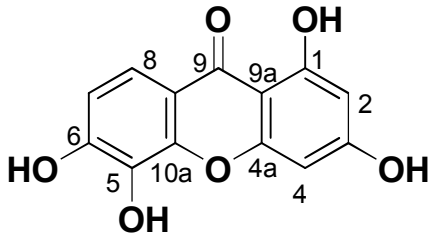


FIGURE 14 Structure of compound **H**

TABLE 13 Comparison of ^{1}H and ^{13}C NMR data of compound **H** with 1,3,5,6-tetrahydroxyxanthone (Farhm; & Chaudhuri. 1979: 2035-2038).

position	δ_H (*mult.*, *J* in Hz)	δ_C	
	Compound **H** ($CDCl_3$ + DMSO-d_6)	1,3,5,6-tetrahydroxyxanthone ($CDCl_3$)	Compound **H** ($CDCl_3$ + DMSO-d_6)
1	12.71 (1H, *s*)	162.94	162.4
2	5.79 (1H, *d*, *J* = 2.0)	97.89	97.2
3		165.14	164.2
4	6.04 (1H, *d*, *J* = 2.0)	93.95	93.4
4a		157.38	156.8
5		132.48	131.5
6		151.92	150.5
7	6.49 (1H, *d*, *J* = 8.7)	113.09	112.0
8	7.14 (1H, *d*, *J* = 8.7)	115.93	115.3
8a		113.09	112.8
9		179.69	179.3
9a		101.48	101.2
10a		146.10	145.3

TABLE 14 ^{1}H, ^{13}C NMR and 2D NMR data of compound **H** (sss4863).

position	δ_H (*mult*., *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	12.71 (1H, *s*)	162.4	C-1, C-2, C-9, C-9a	
2	5.79 (1H, *d*, *J* = 2.0)	97.8	C-1, C-3, C-4, C-9a	
3		165.1		
4	6.04 (1H, *d*, *J* = 2.0)	93.9	C-2, C-3, C-4a, C-9, C-9a	
4a		157.3		
5		1332.4		
6		151.9		
7	6.49 (1H, *d*, *J* = 8.7)	113.0	C-5, C-6, C-8a, C-10a	H-8
8	7.14 (1H, *d*, *J* = 8.7)	115.9	C-5, C-6, C-7, C-9, C-10a	H-7
8a		113.0		
9		179.6		
9a		101.4		
10a		146.1		

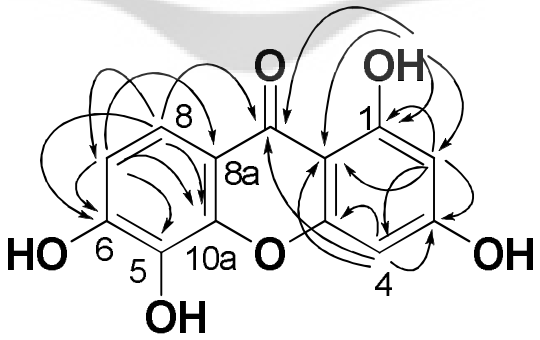


FIGURE 15 Selected HMBC correlations for compound **H**

1.9. Structure determination of compound **I** (isojacareubin)

Compound **I** was obtained as an orange solid, m.p.168-172°C, and gave a molecular ion peak at m/z 325 in the mass spectrum corresponding to the molecular formula $C_{18}H_{14}O_6$. The UV spectrum displayed absorption bands at 256, 300, 329, and 375 nm and its showed in their IR spectra hydroxyl (3318 cm^{-1}), chelated carbonyl (1651 cm^{-1}) and aromatic ring (1620, 1583 cm^{-1}). The ^1H NMR spectrum (Table 15, Figure 16) obtained in acetone-d_6 exhibited two doublets aromatic *ortho* coupled protons at δ 7.00 and 7.63 (2*d, J* = 8.4 Hz, H-7, H-8), singlet at δ 6.14 (H-2) and a chelated hydroxyl at δ 13.31. The signals in the high field region of ^1H NMR spectrum showed the A-ring of compoumd I to be substitution by 2,2-dimethyl pyran ring (δ 7.06, *d, J* = 9.8 Hz, H-11; δ 5.74, *d, J* = 10.0 Hz, H-12; δ 1.46, *s*, H_3-14 and H_3-15), suggesting that compound **I** was isojacareubin. The ^{13}C NMR spectra (Table 15, Figure 16) provided 18 carbons (including two methyls, five methines, ten quaternary carbons and one carboxyl carbon signals). The melting point of compound **H** was 168-172 °C (d), which was compaired to that of the reported isojacareubin (Rath; et al. 1996: 513-520) (m.p. 170-175°C). In the HMBC spectrum (Table 16, Figure 17), the singlet proton at δ_H 13.31 (H-1) showed correlations with C-1, C-2, C-3, C-4, and C-9a, aromatic proton at δ_H 7.63 (H-8) showed correlations with C-6, C-10, and C-9 and methylene protons δ_H 7.06 (H-11) of 2,2-dimethyl pyran ring showed correlations with C-3, C-4a and C-13 confirmed the connection between 2, 2-dimethyl pyran ring was attached to the ring B of xanthone unit. Assignments of ^1H and ^{13}C NMR spectra data of **I** were confirmed by COSY, DEPT, HMQC and HMBC experiments. From the spectroscopic methods and chromatographic comparison with the isojacareubin in several solvent systems, the structure of compound **I** was hence assigned as isojacareubin (**18**).

Isojacareubin was found in *Hypericum* plants such as *H. japonicum* (Ishiguro; et al. 1993: 1583-1535), *H. roeperanum* (Rath; et al. 1996: 513-520). It was reported to exhibit significant antimicrobial activities by the agar-well method using *Staphylococcus aureus*. The compound was examined as a 50% DMSO solution and the activity was expressed by

the inhibitory diameter, which was measured after incubation for 18 hr at 37°. The minimum inhibitory concentration (MIC) of isojacareubin was 125 μg/ml (Ishiguro; et al. 1993: 1583-1535).

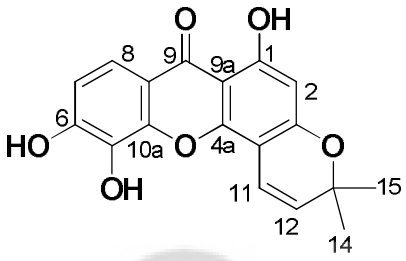


FIGURE 16 Structure of compound **I**

TABLE 15 Comparison of ^{1}H and ^{13}C NMR data of compound **I** (sss4310) with isojacareubin (**18**) (Ishiguro; et al. 1993: 1583-1535).

position	δ_H (*mult.*, *J* in Hz)		δ_C	
	isojacareubin	compound **I**	isojacareubin	compound **I**
1	13.30 (1H, *s*)	13.31 (1H, *s*)	164.3	164.2
2	6.16 (1H, *d*, *J* = 0.7)	6.14 (1H, *s*)	99.5	99.4
3			161.3	161.1
4			103.6	103.5
4a			152.8	152.7
5			133.4	133.3
6			152.8	152.6
7	7.01 (1H, *d*, *J* = 8.6)	7.00 (1H, *d*, *J* = 8.4)	113.9	113.8
8	7.66 (1H, *d*, *J* = 8.6)	7.63 (1H, *d*, *J* = 8.2)	117.7	117.6
8a			114.8	114.6
9			181.4	181.3
9a			102.2	102.0
10a			147.1	147.0
11	7.07 (1H, *dd*, *J* = 0.7, 10.4)	7.06 (1H, *d*, *J* = 9.8)	116.0	115.8
12	5.74 (1H, *d*, *J* = 10.4)	5.74 (1H, *d*, *J* = 10.0)	128.0	128.0
13			78.9	78.8
14, 15	1.48 (6H, *s*)	1.46 (6H, *s*)	28.4	28.3

TABLE 16 ^{1}H, ^{13}C NMR and 2D NMR data of compound **I** (sss4310) in acetone-d_6

position	δ_H (*mult.*, *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
1	13.31 (1H, *s*)	164.2	C-1, C-2, C-3, C-4, C-9a	H-2
2	6.14 (1H, *s*)	99.4	C-1, C-3, C-4, C-9, C-9a	H-1
3		161.1		
4		103.5		
4a		152.7		
5		133.3		
6		152.6		
7	7.00 (1H, *d*, *J* = 8.4)	113.8		H-8
8	7.63 (1H, *d*, *J* = 8.2)	117.6	C-6, C-9, C-10	H-7
8a		114.6		
9		181.3		
9a		102.0		
10a		147.0		
11	7.06 (1H, *d*, *J* = 9.8)	115.8	C-3, C-4a, C-13	
12	5.74 (1H, *d*, *J* = 10.0)	128.0	C-4, C-9a, C-13, C-14, C-15	
13		78.8		
14	1.46 (3H, *s*)	28.3	C-1, C-3, C-12, C-13, C-15	H-12
15	1.46 (3H, *s*)	28.3	C-1, C-3, C-12, C-13, C-14	H-12

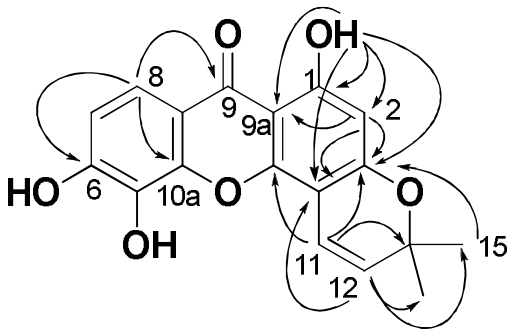


FIGURE 17 Selected HMBC correlations for compound **I**

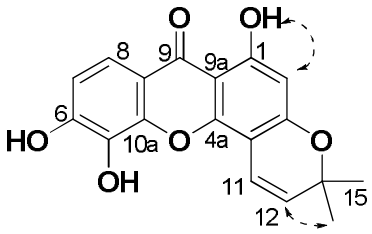


FIGURE 18 Selected NOESY correlations for compound **I**

1.10. Structure determination of compound **J** (morelloflavone, sss4665)

Compound **J** was obtained as a yellow solid and gave an orange coloration with anisaldehyde-H_2SO_4 reagent. On the basis of its ESIMS ($[M-H]^-$ at *m/z* 555), a molecular formula of compound **J** was established as $C_{30}H_{20}O_{11}$ with the support of ^{13}C NMR data. Its IR absorption spectrum showed the presence of hydroxy groups at 3218 cm^{-1}, conjugated γ-pyrone at 1645 cm^{-1} and benzene ring at 1609, 1516 and 1456 cm^{-1}. The UV absorption maxima in MeOH at 222 (shoulder), 254(shoulder), 274(shoulder), 287 and 347 nm were found to belong to a biflavonoid skeleton. The ^1H NMR spectrum (Table 17, Figure 19) of compound **J** showed the signals for biflavonoid mixtures, the major compound of which revealed six aromatic protons doublets, one aromatic proton singlet and the H-2 and H-3 methine protons exhibited at δ_H 5.84 (*d*, *J* = 12.0 Hz), 4.82(*d*, *J* = 12.0 Hz), respectively. The aromatic protons of flavanone part appeared at δ_H 7.14 (*d, J* = 8.1 Hz, 1H), 6.50 (*d*, *J* = 8.1 Hz, 1H), 6.50 (*d*, *J* = 8.3 Hz, 1H) and 7.17 (*br, d*, *J* = 8.3 Hz, 1H), and two protons showed at δ_H 7.14 (*d*, *J* = 8.1 Hz, 1H) and 6.95 (*d*, *J* = 8.1 Hz, 1H), for flavone moiety. Furthermore, the two singlet signals of chelated OH at δ_H 12.15 and 12.77 as singlet and five signals of phenolic OH showed δ_H 8.64, 10.12, 8.83, 10.55, 8.45, as broad singlet. The ^{13}C NMR and DEPT spectra (Table 17, Figure 19) displayed 30 major signals attributable to thirteen methines and seventeen quaternary carbons which were assigned from the ^1H-^1H COSY and ^1H-^{13}C HMQC spectra of compound **J** and comparison with the reported values.

Connections among ring A, B and C of flavanone subgroups were provided by analysis of its HMBC and NOESY spectra (Table 18 and Figures 19 and 20). The NOESY correlations were observed for methine proton at δ_H 5.84 (H-2) to H-6' (δ_H 7.14) of aromatic proton together with HMBC correlations of H-2 to C-1' (δ_C 128.4), C-2' (δ_C 128.1) and C-6' (δ_C 127.8), and the correlations of H-3 to C-2 (δ_C 81.0), C-1'(δ_C 128.4), C-2' (δ_C 128.1) and C-6' (δ_C 127.8) in HMBC spectra, indicating that ring B was connected to ring C. The HMBC correlation of 5-OH, H-6 and H-8 to C-4a (δ_C 101.8) and of 5-OH to C-7 (δ_C 164.0), C-8 (δ_C 96.3) and C-8a (δ_C 166.3) confirmed that ring A connected to ring C.

Connections among ring D, E and F of flavone subgroups were provided by analysis of its HMBC and NOESY spectra. The correlations of H-2''' (δ_H 7.35 *br s*) to C-2'' (δ_C 164.0), C-3''' (δ_C 144.9), C-4''' (δ_C 148.6) and C-6''' (δ_C 118.6) in HMBC spectra together with NOE correlations of H-2''' to H-3'' (δ_H 6.35 *s*) and H-3'' to H-6''' (δ_H 7.14 *d*, *J* = 8.1) indicated that ring E connected to ring F. The HMBC correlations were observed for 5''-OH at δ_H 12.77 *s* to C-4a (δ_C 103.8) and NOE correlations of H-6'' (δ_H 6.33 *s*) to H-3'' (δ_H 6.35 *s*) confirmed that ring D attached to ring F.

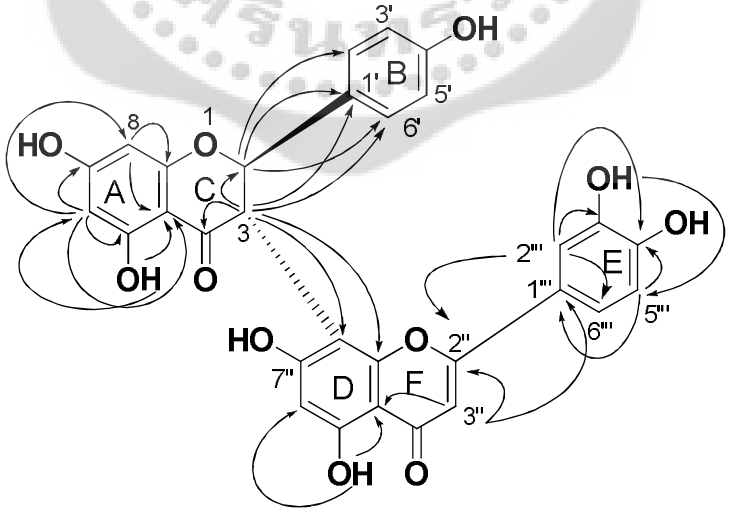


FIGURE 19 Selected HMBC correlations for compound **J**

Connections among flavanone and flavone subgroups were provided by analysis of its HMBC and NOESY spectra. The HMBC correlations were observed for methine proton at δ_H 4.82 (H-3) to C-8'' (δ_C 99.9) and C-8a'' (δ_C 155.4) and NOESY correlations of H-2''' and H-6''' to H-3, of H-6''' to H-2 confirmed that flavone unit was attached to flavanone moiety. On the basis of these data coupled with the compairison of its NMR data with that of morelloflavone, Table 17, the structure of compound **J** was proposed to be morelloflavone (*synonyms* fukugetin).

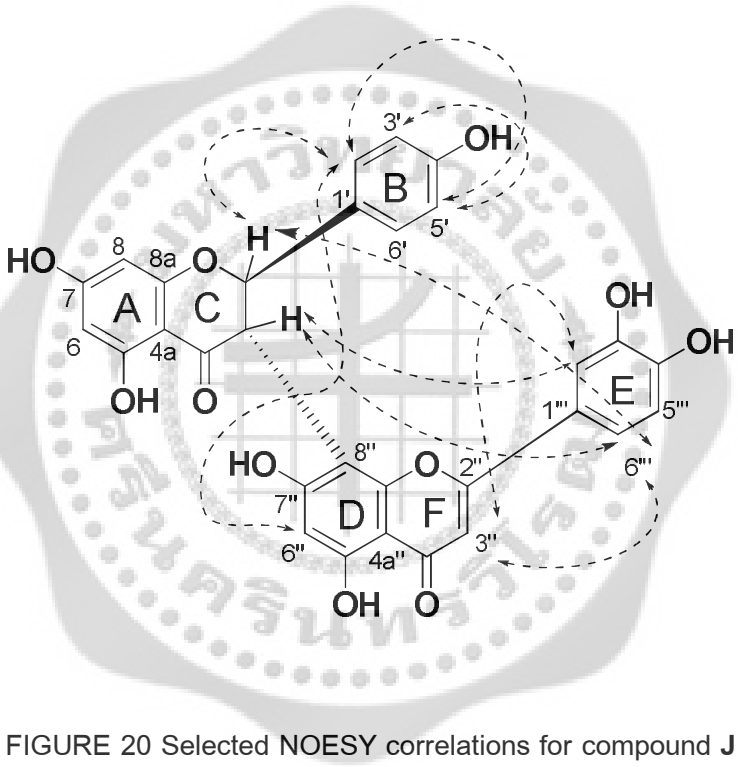


FIGURE 20 Selected NOESY correlations for compound **J**

The stereochemistry at C-2 and C-3 of flavanone unit in conpound **J** were provided by analysis of its ^3J coupling constant value and the large coupling constants (J = 12.0 Hz) of C-2 and C-3 protons in ring C of compound J, in addition, no significant NOE enhancement was observed between both protons, indicated that both hydrogens have a *trans*- diaxial arrangement.

Compairison of the melting points (230-232 ^{o}C, d) and dextrorotatory optical rotation ($[\alpha]_D^{25.8}$ = +161.6 ^{o}) of compound **J** with the reported value of (±) morelloflavone ($[\alpha]_D^{25}$ = 0 ^{o}, m.p. 298-299 ^{o}C, d) and (+) morelloflavone ($[\alpha]_D^{25}$ = +188 ^{o} (Li; et al. 2002: 8709-8717), m.p. 244-245 ^{o}C (d) (Konoshima; et al. 1969; 121-124)), therefore, the structure of **J** was assigned to be (+) morelloflavone. The stereochemistry of (+) morelloflavone was previously assigned as *2R,3S* configurations by its CD spectrum analysis (positive Cotton effect at near 340 and 290 nm) (Li; et al. 2002: 8709-8717). This led to conclude that compound J also has the same *2R,3S* configurations as shown in Figure 20.

Morelloflavone (**19**) (Terashima; et al. 2008: 407-13) was found in *Garcinia* plants such as *G. dulcis* (Roxb.) (Hutadilok; et al. 2007: 655-662) Kurz., *G. livingstonei* (Yang; et al. 2010: 4749-4755), *G. Morella* (Karanjgaokar; et al. 1967: 3195-3198), *G. spicata* Hook. F. (Konoshima; & Ikeshiro. 1969: 121-124). *G. xanthochymus* Hook. f. (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206) and *G. multiflora* Cham. (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206). It was exhibited DPPH and SW-480 colon cancer cells cytoxicity (Baggett; et al. 2005: 354-360) and strong antioxidation (Hutadilok; et al. 2007: 655-662)

TABLE 17 Comparison of ^{1}H and ^{13}C NMR data of compound **J** (sss4665) with **19**

Position	δ_H (*mult.*, *J* in Hz)			δ_C	
	19 (DMSO-d_6)		**J** ($CDCl_3$+DMSO-d_6)	**19** (DMSO-d_6)	**J** ($CDCl_3$+DMSO-d_6)
2	5.71 (*d*, *J* = 1 2.0, 1H)	major	5.84 (*d*, *J* = 12.0, 1H)	81.0	81.0
		minor	5.65 (*d*, *J* = 12.4, 1H)	-	81.8
3	4.89 (*d*, *J* = 1 2.0, 1H)	major	4.82 (*d*, *J* = 12.0, 1H)	48.4	48.9
		minor	4.99 (*d*, *J* = 12.2, 1H)	-	47.8
4	-		-	196.3	196.4
4a	-		-	101.6	101.8
5	-		-	61.8	161.2
6	5.97 (*br s*, 1H)	major	6.06 (*d*, *J* = 3.2, 1H)	95.4	95.4
		minor	-	-	95.5
7	-		-	163.6	164.0
				-	162.9
8	5.97 (*br s*, 1H)	major	6.06 (*d*, *J* = 3.2, 1H)	96.3	96.4
		minor	-	-	96.3
8a	-		-	166.6	166.3
1'	-		-	128.2	128.4
2'	7.15 (*d*, *J* = 8.3, 1H)	major	7.17 (*d*, *J* = 8.1, 1H)	128.6	128.1
		minor	7.18 (*br d*, *J* = 8.1, 1H)	-	-
3'	6.39 (*d*, *J* = 8.3, 1H)	major	6.50 (*d*, *J* = 8.3, 1H)	114.5	114.6
		minor	6.67 (*d*, *J* = 8.3, 1H)		-
4'	-		-	157.4	156.9
5'	6.39 (*d*, *J* = 8.3, 1H)		6.50 (*d*, *J* = 8.3, 1H)	114.5	114.6
			6.67 (*d*, *J* = 8.3, 1H)	-	-
6'	7.15 (*d*, *J* = 8.3, 1H)		7.14(*d*, *J* = 8.1, 1H)	128.6	127.8
			7.06(*br d*, *J* = 8.1, 1H)	-	-
2''	-		-	163.8	163.4
3''	6.58 (*s*, 1H)		6.35 (*s*, 1H)	102.3	102.8
4''	-		-	181.7	182.0
4a''	-		-	103.2	103.8
5''	-		-	160.6	160.6
6''	6.23 (*s*, 1H)	major	6.33 (*s*, 1H)	98.7	98.9
		minor		-	98.5
7''	-		-	162.9	161.0
8''	-		-	100.6	99.9
8a''	-		-	155.3	155.4
1'''	-		-	121.1	122.2
2'''	7.42 (*br s*, 1H)		7.35 (*br s*, 1H)	113.4	112.9
3'''	-		-	145.7	144.9
4'''	-		-	49.8	148.6
5'''	6.91 (*d*, *J* = 8.1, 1H)		6.95 (*d*, *J* = 8.1, 1H)	116.2	115.4
6'''	6.97 (*d*, *J* = 8.0, 1H)		7.14 (*d*, *J* = 8.1, 1H)	119.4	118.6
5-OH	12.25 (*s*, 1H)		12.15, 12.11 (*s*, 1H)	-	-
7-OH	-		10.12 (*br s*, 1H)	-	-
4'-OH	-		8.83 (*br d*, 1H)	-	-
5''-OH	13.07 (*s*, 1H)		12.77, 12.68 (*s*, 1H)	-	-
7''-OH	-		10.55 (*br s*, 1H)	-	-
3'''-OH	-		8.64 (*s*, 1H)	-	-
4'''-OH	-		8.45 (*br s*, 1H)	-	-

TABLE 18 ^{1}H, ^{13}C NMR and 2D NMR data of compound **J** (sss4665)

Position	δ_H (*mult.*, *J* in Hz)	δ_C	HMBC correlations	NOESY correlations
2	5.84 (*d*, *J* = 12.0,	81.8, 81.0	C-1', C-2', C-6'	H-3 (weak), H-6', H-6'''
3	4.99 (*d*, *J* = 12.2)	48.9, 47.8	C-2, C-4, C-1', C-2', C-6', C-8'', C-8a''	H-2 (weak), H-6', H-2''', H-6'''
4	-	196.4	-	-
4a	-	101.8	-	-
5	-	161.2	-	-
6	6.06 (*d*, *J* = 3.2, 1H)	95.4, 95.5	C-4a, C-7, C-8	
7	-	164.0,	-	-
8	6.06 (*d*, *J* = 3.2, 1H)	96.4, 96.3	C-4a, C-6, C-7, C-8a	
8a	-	166.3	-	-
1'	-	128.4	-	-
2'	7.17 (*br. d*)	128.1,	H-1', H-4', H-6'	H-2, H-3, H-3', H-5'
3'	6.67 (*d*, *J* = 8.3, 1H)	114.6	H-1', H-2', H-5', H-4'	H-2', H-6'
4'	-	156.9	-	-
5'	6.67 (*d*, *J* = 8.3, 1H)	114.6	H-1', H-3', H-4', H-6'	H-2', H-3', H-6'
6'	7.14 (*d*, *J* = 8.1, 1H) 7.06(*d*, J = 8.1, 1H)	127.8	H-1', H-2', H-4'	H-2 (weak), H-3, H-2', H-3', H-5'
2''	-	164.0,	-	-
3''	6.35 (*s*, 1H)	102.8,	H-4a'', H-2'', H-1'''	H-6'', H-2''', H-6'''
4''	-	182.0	-	-
4a''	-	103.8	-	-
5''	-	160.6	-	-
6''	6.33 (*s*, 1H)	98.9, 98.5	H-5'', H-4a'', H-7'', H-8''	H-3''
7''	-	161.0	-	-
8''	-	99.9	-	-
8a''	-	155.4	-	-
1'''	-	122.3	-	-
2'''	7.35 (*br. s*, 1H)	112.9	C-2'', C-3''', C-4''', C-6'''	H-3, H-3'', H-6'''
3'''	-	144.9	-	-
4'''	-	148.6	-	-
5'''	6.95 (*d*, *J* = 8.3, 1H)	155.4	C-1''', C-3''', C-4'''	H-6'''
6'''	7.14 (*d*, *J* = 8.1, 1H)	118.6	C-2''', C-4'''	H-2, H-3, H-3'', H-5'''
5-OH	12.15, 12.11 (*s*, 1H)	-	C-4a, C-7, C-8, C-8a	-
7-OH	10.12 (*br. s*, 1H)	-	-	-
4'-OH	8.83 (*br. d*, 1H)	-	-	-
5''-OH	12.77, 12.68 (*s*, 1H)	-	C-4a'' , C-5''(OH), C-6'', C-7''	-
7''-OH	10.55 (*br. s,* 1H)	-	-	-
3'''-OH	8.64 (*s*, 1H)	-	-	-
4'''-OH	8.45 (*br. s*, 1H)	-	-	-

1.11. Structure determination of compound **K** (vokensiflavone, SSS4547)

Compound **K** was obtained as a yellow solid, and gave an orange coloration same as that of compound **J** which was indicated that **J** was also a biflavonoid. On the basis of its ESIMS ($[M-H]^-$ at *m/z* 539), a molecular formula of compound **K** was established as $C_{30}H_{20}O_{10}$ which was 16 mass units lesser than that of compound **J**. This indicated that **K** has one hydroxyl group less than that of compound **J**. Its IR spectrum exhibited absorption bands for hydroxyl (3184 cm^{-1}), chelated γ-pyrone (1634 cm^{-1}) and aromatic ring (1506 cm^{-1}) and showed UV absorption maxima in MeOH at 221(sholder), 289, 325 and 342 nm which were very similar to those of compound **J**. The ^{1}H, ^{13}C NMR and Dept spectra (Table 19, Figure 21) indicated for the presence of a biflavonoid skeleton in compound **K**. Its ^{1}H NMR spectra was similar to those of compound **J** (morelloflavone) except that compound **K** showed 14 methines and 16 quaternary carbons whereas compound **J** showed 13 methines and 17 quaternary carbons. The NMR data also supported that compound **K** has one hydroxyl group less than that of compound **J**. Compound **K** showed the signals of biflavonoid mixtures, the major compound revealed 8 proton doublets, the H-2 and H-3 methine protons exhibited at δ_H 5.85 (*d*, *J* = 12.0 Hz) and 4.75 (*d*, *J* = 12.0 Hz), respectively. The aromatic protons of flavanone part appeared at δ_H 7.07 (*d*, *J* = 9.3 Hz, 1H), 6.50 (*d*, *J* = 7.8 Hz, 1H), 6.50 (*d*, *J* = 7.8 Hz, 1H) and 7.07 (*d*, *J* = 9.3 Hz, 1H), and two group doublets aromatic *ortho* coupled protons at δ_H 7.51, 6.74 (d, *J* = 8.6 Hz, H-2''' and H-3''') and δ_H 6.74, 7.51 (d, *J* = 8.6 Hz, H-5''' and H-6''') for flavone moiety. Furthermore, the two singlet signals of chelated OH apperares at δ_H 12.28, 12.90 as singlet.

Connections among rings A, B and C of flavanone subgroup were provided by analysis of its HMBC and NOESY spectra (Table 20 and Figures 21 and 22). The NOESY correlations were observed for methine proton at δ_H 5.85 (H-2) to H-6' (δ_H 7.07, *d*) of aromatic protons together with HMBC correlations of H-2 to C-1' (δ_C 128.6), C-2' (δ_C 128.4) and C-6' (δ_C 128.4), and the correlations of H-3 to C-2 (δ_C 81.0), C-1' (δ_C 128.6), C-2' (δ_C 128.4) and C-6' (δ_C 128.4) in HMBC spectra, indicated that ring B was connected to ring C. The HMBC correlation of 5-OH, H-6 and H-8 to C-4a (δ_C 102.0) and H-8 to C-8a (δ_C 163.1) confirmed that ring A connected to ring C.

Connections among rings D, E and F of flavone subsgroup were provided by analysis of its HMBC and NOESY spectra. The correlations of H-2''' (δ_H 7.51 d) to C-4''' (δ_C 161.0) and C-6''' (δ_C 127.9) in HMBC spectra together with NOESY correlations of H-2''' to H-3'' (δ_H 6.35 *s*) and H-3'' to H-6''' (δ_H 7.51 *d*, *J* = 8.5) indicated that ring E connected to ring F. The HMBC correlations were observed for 5''-OH at δ_H 12.90 *s* to C-4a'' and NOE correlations of 5''-OH to H-6'' (δ_C 99.2) confirmed that ring D connected to ring F.

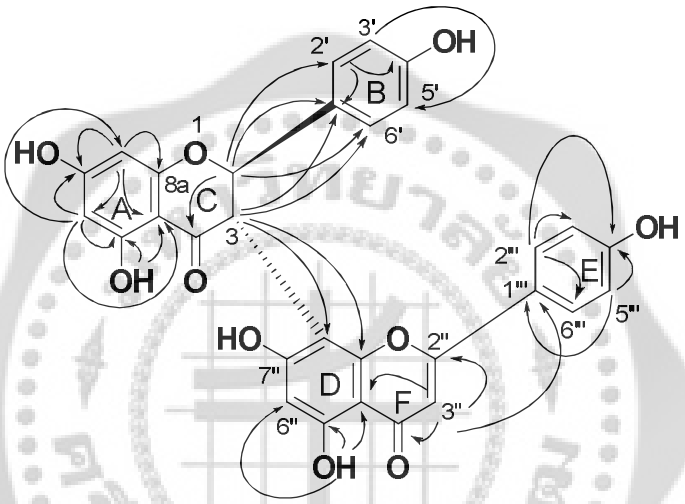


FIGURE 21 Selected HMBC correlations for compound **K**

Connections among flavanone and flavone subsgroups were provided by analysis of its HMBC and NOESY spectra. The HMBC correlations were observed for methine proton at δ_H 4.75 (H-3) to C-8'' (δ_C 101.1) and C-8a'' (δ_C 155.3) and NOESY correlations of H-2''' and H-6''' to H-3, indicating that flavone unit was attached to flavanone moiety. The result indicates that of the structure of compound **K** was proposed to be vokensiflavone.

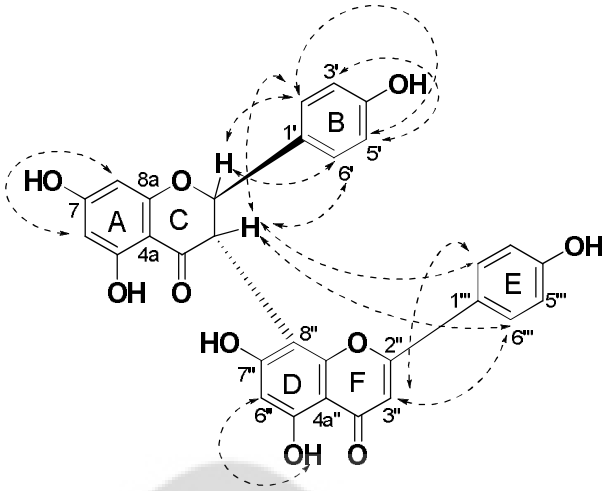


FIGURE 22 Selected NOESY correlations for compound **K**

The stereochemistry at C-2 and C-3 of flavanone unit in conpound **K** were provided by analysis of its ^{3}J coupling constant value. The large coupling constants (J = 12.0 Hz) of C-2 and C-3 protons in ring C of compound **K**, in addition, no significant NOE enhancement was observed between both protons, indicated that both hydrogens have a *trans*- diaxial arrangement.

Compound **K** gave m.p. at 220-221°C (d) and dextrorotatory optical rotation, $[\alpha]_D^{25.8}$ = +142.0. Comparison these physical data with that of (±) vokensiflavone, $[\alpha]_D^{15}$ = 0 °, m.p. 290-293 °C (d) (Konoshima; & Ikeshiro. 1969; 121-124) and (+) vokensiflavone-7-sulfate, $[\alpha]_D^{25}$ = +113 (Li; et al. 2002: 8709-8717)], therefore, the structure of **J** was assigned to be (+) vokensiflavone. (+) Vokensiflavone-7-sulfate was previously assigned as *2R,3S* configurations by its CD spectrum analysis (positive Cotton effect at near 340 and 290 nm). This led to conclude that compound **K** also has the same *2R,3S* configurations as shown in Figure 22.

Vokensiflavone (**20**) was found in *Garcinia* plants such as *G. livingstonei* (Yang; et al. 2010: 4749-55), *G. xanthochymus* (Baggett; et al. 2005: 354-360), *G. spicata* Hook. F. (Konoshima; & Ikeshiro. 1969: 121-124) and *G. xanthochymus* Hook. f. (Konoshima; Ikeshiro; & Miyahara. 1970: 1203-1206). It was reported to exhibit significantant DPPH and SW-480 colon cancer cells cytoxicity (Baggett; et al. 2005: 354-360).

TABLE 19 Comparison of ^{1}H and ^{13}C NMR data of compound **K** (SSS4547) with vokensiflavone (Chen; et al. 1957: 300-303).

Position	δ_H (*mult.*, *J* in Hz)			δ_C		
	vokensiflavone[a]	compound **K**[b]		vokensiflavon[a]	compound **K**[b]	
		major	minor		major	minor
2	5.80 (1H, *d*, *J* = 12.0)	5.85 (1H, *d*, *J* = 12.0)	5.62 (1H, *d*, *J* = 12.0)	81.4	81.0	82.0
3	4.90 (1H, *d*, *J* = 12.0)	4.75 (1H, *d*, *J* = 12.0)	4.98 (1H, *d*, *J* = 12.0)	48.2	49.2	48.0
4	-		-	196.6	196.3	
4a	-		-	101.7	102.0	
5	-		-	163.7	163.7	
6	6.22 (1H, *br s*)	6.05 (1H, *br d*, *J* = 3.0)		96.4	96.6	96.7
7	-		-	166.6	166.6	166.9
8	6.30 (1H, *d*, *J* = 2)	6.05 (1H, *br d*, *J* = 3.0)		95.3	95.6	95.7
8a	-		-	163.8	163.1	
1'	-		-	128.1	128.6	
2'	7.13 (1H, *d*, *J* = 9)	7.07 (1H, *d*, *J* = 9.3)	7.08 (1H, *d*, *J* = 8.7)	128.1	128.4	
3'	6.63 (1H, *d*, *J* = 9)	6.50 (1H, *d*, *J* = 7.8)	6.53 (1H, *d*, *J* = 8.3)	114.6	114.9	
4'	-		-	162.2	161.2	
5'	6.63 (1H, *d*, *J* = 9)	6.50 (1H, *d*, *J* = 7.8)	6.53 (1H, *d*, *J* = 8.3)	114.6	114.9	
6'	7.13 (1H, *d*, *J* = 9)	7.06 (1H, *d*, *J* = 9.3)	7.08 (1H, *d*, *J* = 8.7)	128.1	128.4	
2''	-		-	163.7	162.6	
3''	6.50 (1H, *s*)	6.35 (1H, *s*)		102.8	102.9	
4''	-		-	181.6	182.2	
4a''	-		-	103.6	104.0	
5''	-		-	160.4	161.0	
6''	6.22 (1H, *s*)	6.35 (1H, *s*)		98.5	99.2	98.7
7''	-		-	162.8	164.2	
8''	-		-	100.6	100.1	
8a''	-		-	155.3	157.3	
1'''	-		-	121.3	121.7	
2'''	7.70 (1H, *d*, *J* = 9)	7.51 (1H, *d*, *J* = 8.5)	6.74 (1H, *d*, *J* = 8.7)	128.1	127.7	
3'''	6.68 (1H, *d*, *J* = 9)	7.61 (1H, *d*, *J* = 8.6)	6.96 (1H, *d*, *J* = 8.5)	115.9	116.0	
4'''	-		-	161.0	161.6	
5'''	6.87 (1H, *d*, *J* = 9)	6.74 (1H, *d*, *J* = 8.6)	6.96 (1H, *d*, *J* = 8.5)	115.9	116.0	
6'''	7.70 (1H, *d*, *J* = 9)	7.51 (1H, *d*, *J* = 8.5)	7.61 (1H, *d*, *J* = 8.7)	128.1	127.9	
5-OH	12.40 (*s*)	12.28 (1H, *s*)		-	-	
7-OH	-		-	-	-	
4'-OH	-		-	-	-	
5''-OH	13.27 (*d*)	12.90 (1H, *s*)	12.85 (1H, *s*)	-	-	
7''-OH	-		-	-	-	
3'''-OH	-		-	-	-	
4'''-OH	-		-	-	-	

[a] in DMSO-d_6

[b] in $CDCl_3$ + DMSO-d_6

TABLE 20 ^{1}H, ^{13}C NMR and 2D NMR data of compound **K** (SSS4547).

Position	δ_H (*mult.*, *J* in Hz)		δ_C		HMBC correlations	NOESY correlations
	major	minor	major	minor		
2	5.85 (1H, *d*, *J* = 12.0)		81.0	82.0	C-4, C-3, C-1', C-2', C-6'	H-3, H-2', H-6'
3	4.75 (1H, *d*, *J* = 12.0)	4.98 (1H, *d*, *J* = 12.0)	49.2	48.0	C-2, C-4, C-1', C-2', C-6', C-2''C-8'', C-8a''	H-2, H-2', H-6', H-2''', H-6'''
4	-	-	196.3	196.7	-	-
4a	-	-	102.0		-	-
5	-	-	163.7		-	-
6	6.05 (1H, *br d*, *J* = 3.0)	-	96.6	96.7	C-4a, C-5, C-7, C-8, C8a	H-8
7	-	-	166.6	166.9	-	-
8	6.05 (1H, *br d*, *J* = 3.0)	-	95.6	95.7	C-4a, C-6, C-7, C-8a	H-6, H-5(OH)
8a	-	-	163.1		-	-
1'	-	-	128.6		-	-
2'	7.07 (1H, *d*, *J* = 9.3)	7.08 (1H, *d*, *J* = 8.7)	128.4		C-2, C-1', C-3', C-4', C6'	H-2, H-3, H-3'
3'	6.50 (1H, *d*, *J* = 7.8)	6.53 (1H, *d*, *J* = 8.3)	114.9		C-1', C-2', C-4', C-5', C6'	-
4'	-	--	161.2		-	-
5'	6.50 (1H, *d*, *J* = 7.8)	6.53 (1H, *d*, *J* =8.3)	114.9		C-1', C-2', C-3', C-4', C6'	-
6'	7.06 (1H, *d*, *J* =9.3)	7.08 (1H, *d*, *J* =8.7)	128.4		C-2, C-1', C-4'	H-2, H-3, H-5'
2''	-	-	162.6		-	-
3''	6.35 (1H, *s*)	-	102.9		C-2'', C-4'', C-4a'' C-5''', C-1''', C6'	H-2''', H-6'''
4''	-	-	182.2		-	-
4a''	-	-	104.0		-	-
5''	-	-	161.0		-	-
6''	6.35 (1H, *s*)	-	99.2	98.7	C--4a'' C-5'', C-7', C-8'''	H-5''(OH)
7''	-	-	164.2		-	-
8''	-	-	100.1		-	-
8a''	-	-	155.3		-	-
1'''	-	-	121.7		-	-
2'''	7.51 (1H, *d*, *J* =8.5)	6.74 (1H, *d*, *J* =8.7)	127.7		C-1''', C-2''', C-3''', C-4''', C-6'''	H-3, H-5(OH), H-3'', H-3'''
3'''	7.61 (1H, *d*, *J* =8.6)	6.69 (1H, *d*, *J* =8.5)	116.0		C-1'', C-2''', C-5'''C-4'''	-
4'''	-	-	161.6		-	-
5'''	6.74 (1H, *d*, *J* =8.6)	6.96 (1H, *d*, *J* =8.5)	116.0		C-1''', C-2''', C-3''', C-4'''	-
6'''	7.51 (1H, *d*, *J* =8.5)	7.61 (1H, *d*, *J* =8.7)	127.9		C-1''', C-2''', C-3''', C-4''', C-6'''	H-3'', H-5'''
5-OH	12.28 (1H, *s*)	-	-		C-4a, C-5, C-8, C-8a	H-6, H-2'''
7-OH	-	-	-		-	-
4'-OH	-	-	-		-	-
5''-OH	12.90 (1H, *s*)	12.85 (1H, *s*)	-		C-4a'' , C-5''(OH), C-6''	H-6''
7''-OH	-	-	-		-	-
3'''-OH	-	-	-		-	-
4'''-OH	-	-	-		-	-

1.12. Structure determination of compound **L** (mixture of rubraxanthone and cowaxanthone, sss4868)

Compound **L** was obtained as a yellow solid and its ^{1}H NMR spectrum (Table 21, Figure 23) indicated the presence of two xanthone skeletons as in compound **L** with only one geranyl group at C-2 or C-8 in the molecule. The ^{1}H-NMR spectra of the first group was similar to those of compound C (cowaxanthone). The last group showed the ^{1}H-NMR spectra was similar to those of compound B (cowanin) but compound L had an additional isolated aromatic proton at δ 6.12 (*d*, *J*= 2.3) was assigned at C-2 and the lack of a prenyl unit. From the NMR spectrum pattern and chromatographic comparison with the authentic mixture of rubraxanthone (**12**) and cowaxanthone (**9**) (in ration 1:0.2) in several solvent systems, the structure of compound **L** was identified as rubraxanthone (**12**) and cowaxanthone (**9**).

Rubraxanthone (**12**) was found in *Garcinia* plants such as *G. cowa* (Limnusont. 2007: 49-52), G. *fusca* (Ito; et al. 2003: 200-205) and *G. merguensis* Wight (Kijjoa; et al. 2008: 864-866) *G. parvifolia* Miq. (Ee; et al. 2009: 105-110). It was investigated for their inhibitory effects on platelet activating factor (PAF) binding to rabbit platelets using 3H-PAF as a ligand, which it showed a strong inhibition with IC_{50} value of 18.2 µgM (Jatan; et al. 2002: 1133-1134). The results of cytotoxicity evaluation showed that rubraxanthone were inhibitory to L1210 cells, with IC_{50} values in the range of 3 to 8 µg/mL (Kardono; et al. 2006: 483-486).

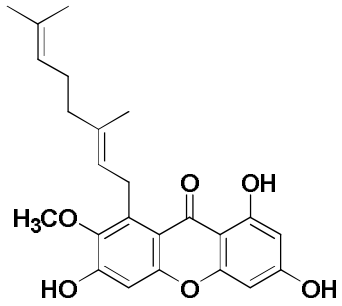


FIGURE 23 Structure of compound **12**

TABLE 21 Comparison of ^{1}H and ^{13}C NMR data of compound **L** (sss4868) with rubraxanthone (**12**) (Limnusont. 2007: 49-52.) and cowaxanthone (**9**).

position	δ_H (*mult.*, *J* in Hz)		δ_H (*mult.*, *J* in Hz)	
	cowaxanthone ($CDCl_3$)	rubraxanthone (acetone-d_6)	compound **L** (cowaxanthone)	compound **L** (rubraxanthone)
1	13.41 (1H, s)	13.50 (1H, s)	.31 (1H, s)	13.36 (1H, s)
2	-	6.18 (1H, *s*)		6.12 (2H, *d*, *J* = 2.3)
4	6.46 (1H, *s*)	6.30 (1H, *s*)	6.33 (1H, s)	6.19 (1H, *d*, *J* = 2.3)
5	6.89 (1H, *s*)	6.83 (1H, *s*)	6.82 (1H, s)	6.75 (1H, s)
8	7.53 (1H, *s*)	-	7.50 (1H, s)	-
11	3.35 (2H, *d*, *J* = 7.0)	4.11 (2H, *br d*, *J* = 5.7)	3.38 (2H, *d*, *J* = 6.9)	3.98 (2H, *d*, *J* = 5.6)
12	5.30 (1H, *br t*, *J* = 7.2)	5.26 (1H, *br t*, *J* = 5.7)	5.18 (1H, *br t*, *J* = 6.2)	5.18 (1H, *br t*, *J* = 6.2)
14, 15	1.95 (4H, *m*)	2.04 (4H, *m*)	2.00 (4H, *m*)	2.00 (4H, *m*)
	1.95 (4H, *m*)	2.04 (4H, *m*)	2.00 (4H, *m*)	2.00 (4H, *m*)
16	5.06 (1H, *br t*, *J* = 7.2)	5.02 (1H, *br t*, *J* = 5.7)	4.95 (1H, *br t*)	4.95 (1H, *br t*)
18	1.58 (3H, *s*)	1.54 (3H, *s*)	1.49 (3H, *s*)	1.49 (3H, s)
19, 20	1.78 (3H, *s*)	1.82 (3H, *s*)	1.77 (3H, *s*)	1.77 (3H, s)
	1.53 (3H, *s*)	1.51 (3H, *s*)	1.58 (3H, *s*)	1.58 (3H, s)
7-OCH_3	3.96 (3H, *s*)	3.78 (3H, s)	3.91 (3H, *s*)	3.91 (3H, s)

2. Structure determination of compounds isolated from the ethyl acetate extract from the fresh green fruit root of *G. fusca*

2.1. Structure determination of compound **M** (α-mangostin, sss4609)

Compound **M** was the major xanthone obtained as a yellow solid. From the NMR spectrum pattern and chromatographic comparison with the authentic α-mangostin in several solvent systems, the structure of compound **M** was identified as α-mangostin (**13**).

2.2. Structure determination of compound **N** (β-mangostin, sss4474)

Compound **N** was obtained as a yellow solid. From the NMR spectrum pattern and chromatographic comparison with the authentic β-mangostin in several solvent systems, the structure of compound **N** was identified as β-mangostin (**10**).

2.3 Structure determination of compound **O** (cowanin, sss4652)

Compound **O** was obtained as a yellow solid. From the NMR spectrum pattern and chromatographic comparison with the authentic cowanin in several solvent systems, the structure of compound **O** was identified as cowanin (**11**).

2.4. Structure determination of compound **P** (cowaxanthone, sss4777)

Compound **P** was obtained as a yellow solid. From the NMR spectrum pattern and chromatographic comparison with the authentic cowaxanthone in several solvent systems, the structure of compound **P** was identified as cowaxanthone (**9**).

2.5. Structure determination of compound **Q** (cowanol, sss4528)

Compound **Q** was obtained as an orange solid. From the NMR spectrum pattern and chromatographic comparison with the authentic cowanol in several solvent systems, the structure of compound **Q** was identified as cowanol (**14**).

2.6. Structure determination of compound **R** (fuscaxanthone A, sss43328)

Compound R was obtained as an orange solid, and was less polar than compound B (R_f value of 0.48). The peak at m/z 476 in its ESMS data was compatible with the molecular formula $C_{29}H_{34}O_7$. The UV spectrum of R exhibited characteristic absorption bands of a xanthone ($\lambda_{\max}^{MeOH}$, 211, 289, 325 and 342 nm) and Its IR spectrum exhibited apsorption bands for hydroxyl (3184 cm^{-1}), chelated carbonyl (1634 cm^{-1}) and aromatic ring (1506 cm^{-1}). The ^{1}H NMR spectra (Table 22, Figure 24) was almost indentical to that of compound **B** (cowanin) except that a prenyl unit at C-2 and a phenolic hydroxyl at C-3 were replaced by a 2,2-dimethylpyran ring and addition two doublet signals of *ortho* protons δ 3.48 and 5.47 (*br d*, *J* = 7.7 Hz, H-11 and H-12). Orther signal of compound **R** was the similar compound **B**, which showed the presence of a chelated phenolic hydroxyl group of 1-OH at δ 13.96, a singlet resonance of methoxy group at δ 3.80 (7-OCH_3) and two singlet signals of two isolated aromatic protons H-4 and H-5 at δ 6.30 and 6.80, respectively. The signals of the geranyl unit appeared as follow: two olefinic protons at δ 5.24 (H-17) and 5.00 (H-21), three sets of methylene groups at δ 4.09 (H-16), 2.03 (H-19 and H-20) and three singlets vinylic methyl groups at δ 1.79 (H-24), 1.59 (H-23) and 1.54 (H-25). From the NMR spectrum pattern and chromatographic comparison with the authentic fuscaxanthone A in several solvent systems, thus compound **R** was elucidated as fuscaxanthone A.

Fuscaxanthone A (**1**) was found in plants including *Garcinia* such as *G. cowa* (Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153), *G. fusca* (Ito; et al; 2003, 200-205) and It was reported to exhibit significant radical scavenging activity (Mahabusarakaml; Chairerk; & Taylor. 2005: 1148-1153).

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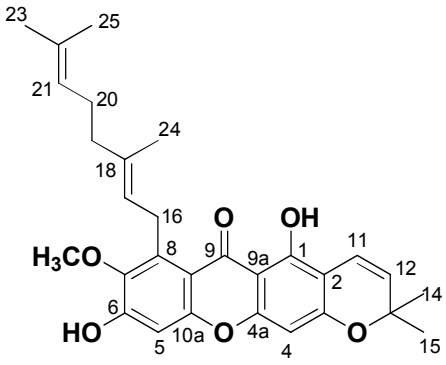

FIGURE 24 Structure of compound **R**

TABLE 22 Comparison of ^{1}H NMR data of compound **R** (sss3328) with fuscaxanthone A (**1**) (Ito; et al. 2003: 200-205).

position	δ_H (*mult.*, *J* in Hz)	
	fuscaxanthone **A** ($CDCl_3$)	compound **R**
1	13.70 (*s*)	13.67 (1H, *s*)
4	6.24 (*s*)	6.22 (1H, *s*)
4a		
5	6.83 (*s*)	6.81 (1H, *s*)
11	6.72 (*d*, *J* = 10.0)	6.70 (1H, *d*, *J* = 10.1)
12	5.56 (*d*, *J* = 10.0)	5.44 (1H, *d*, *J* = 10.1)
14	1.46 (3H, *s*)	1.44 (3H, *s*)
15	1.46 (3H, *s*)	1.44 (3H, *s*)
16	4.09 (2H, *d*, *J* = 7.3)	4.06 (2H, *d*, *J* = 6.3)
17	5.26 (*m*)	5.32 (1H, *br t, J* = 6.3)
18		
19, 20	2.01 (2H, *m*)	1.97 (2H, *m*)
	2.04 (2H, *m*)	1.97 (2H, *m*)
21	5.02 (m)	5.00 (1H, *br t*, *J* = 6.3)
23	1.54 (3H, *s*)	1.57 (3H, *s*)
24	1.80 (3H, *s*)	1.80 (3H, *s*)
25	1.52 (3H, *s*)	1.67 (3H, *s*)
7-OCH_3	3.80 (3H, *s*)	3.78 (3H, *s*)

CHAPTER 5

CONCLUSION

Investigation of the chemical constituents of the root of *G. fusca* led to the isolation of eight known xanthones named, α-mangostin (**13**), β-mangostin (**10**), cowanin (**11**), cowaxanthone (**9**), cowanol (**14**) fuscaxanthone G (**7**), 1,3,5,6,-tetrahydroxyxanthone (**17**) isojacareubin (**18**) and a mixture of rubraxanthone (**12**) and cowaxanthone (**9**), together with two known biflavonoids namely, morelloflavone (**19**) and vokensiflavone (**20**) and one triterpene named β-sitosterol (**21**) from the root of this plant. This is the first report on isolation of compounds **17-20** from this plant. The structures of known triterpene, xanthones and biflavonoids were elucidated by spectroscopic techniques, whilst the known compounds were identified by comparisons of spectroscopic data with those of reported values and chromatographic comparison with authentic samples in several solvent systems.

Investigation of the chemical constituents of the fresh green fruit of *G. fusca* led to the isolation of six xanthones, α-mangostin (**13**), β-mangostin (**10**), cowanin (**11**), cowaxanthone (**9**), cowanol (**14**) and fuscaxanthone A (**1**). The structures of all compounds were elucidated by spectroscopic techniques, especially 1D and 2D NMR and MS including by comparison of their spectroscopic data with those reported in the literature.

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APPENDIX

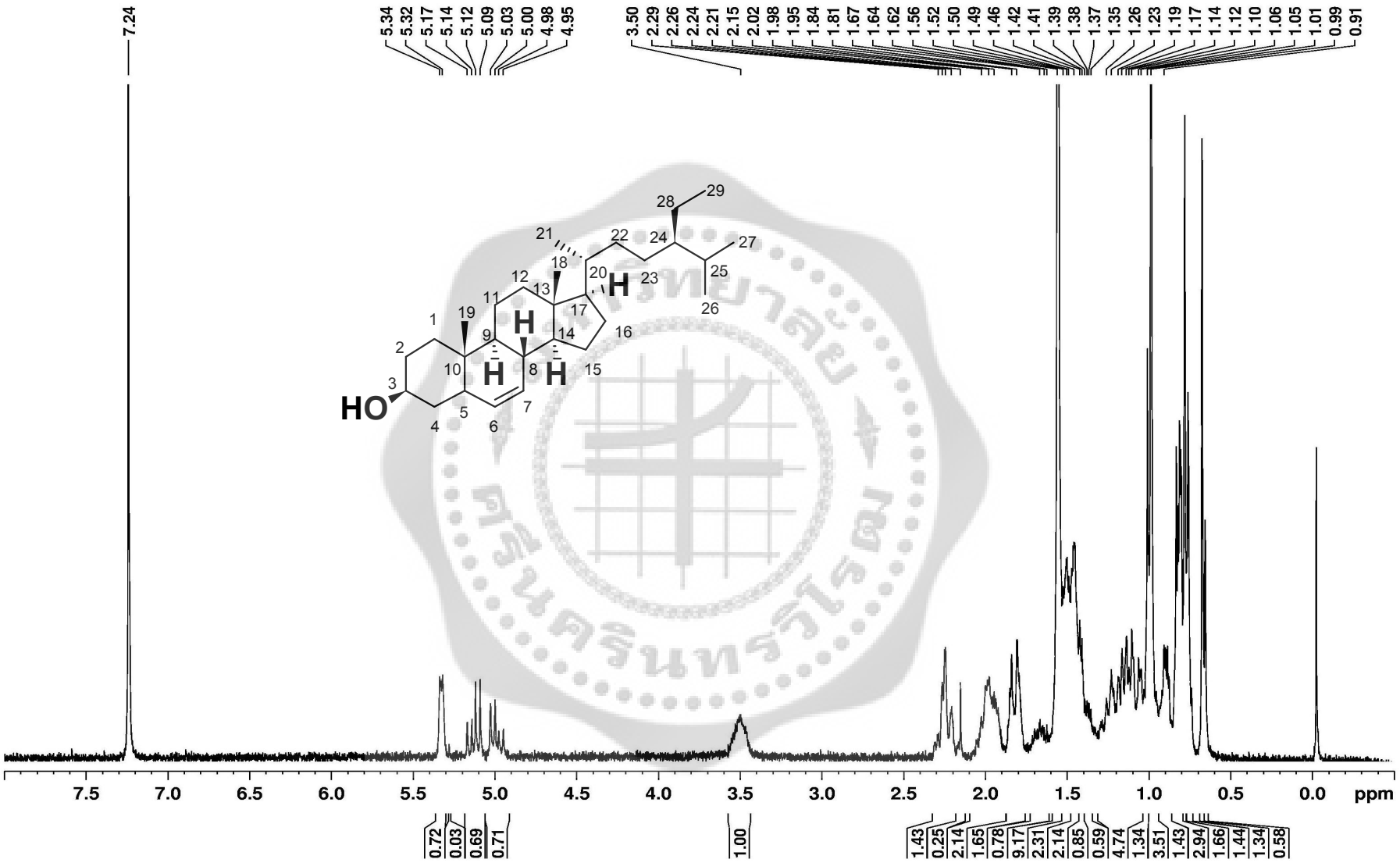

Figure 25 ^{1}H NMR of compound A (β-sitrosterol, sss4192) in $CDCl_3$

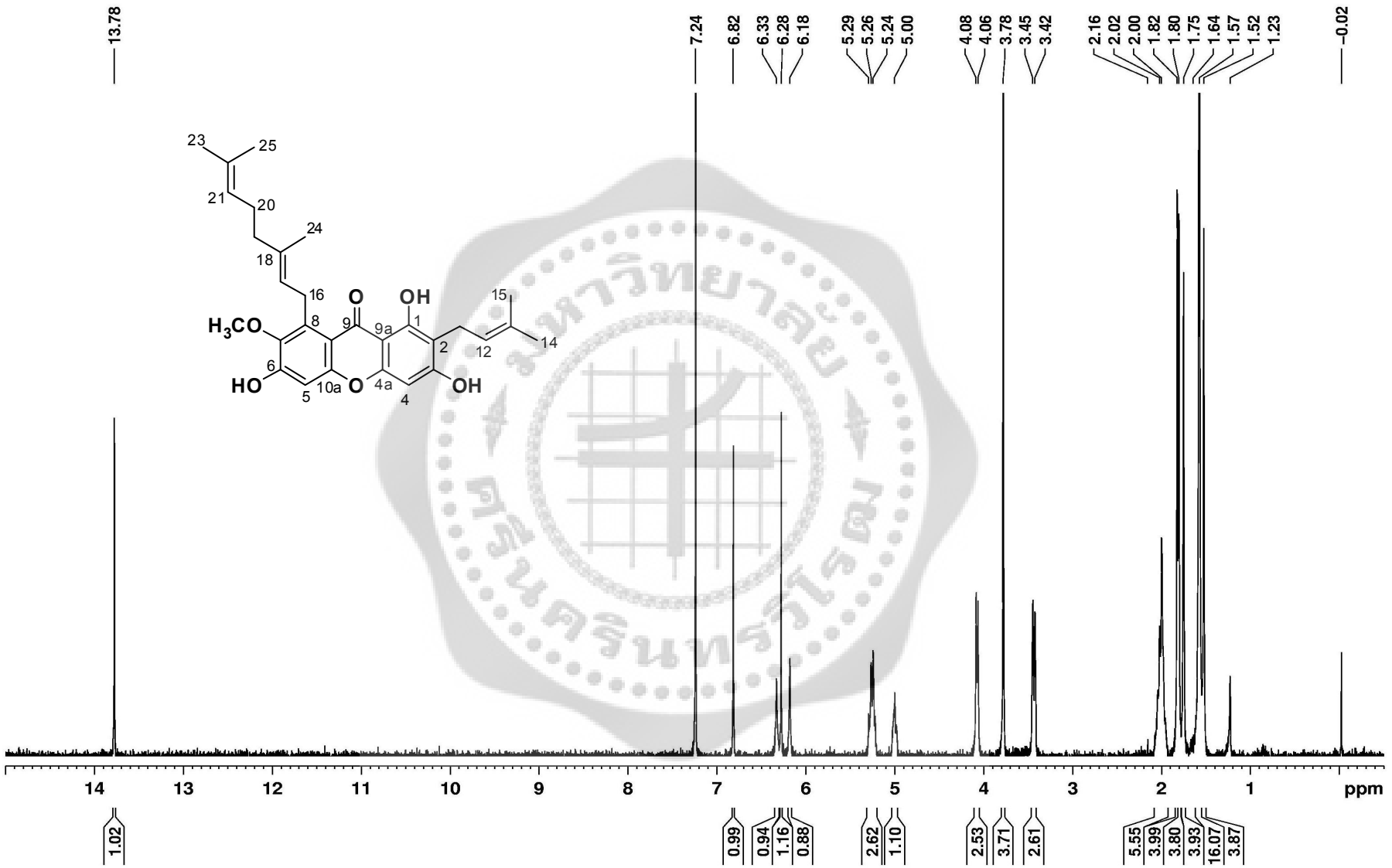

Figure 26 ^{1}H NMR of compound B (cowanin, sss4099) in $CDCl_3$

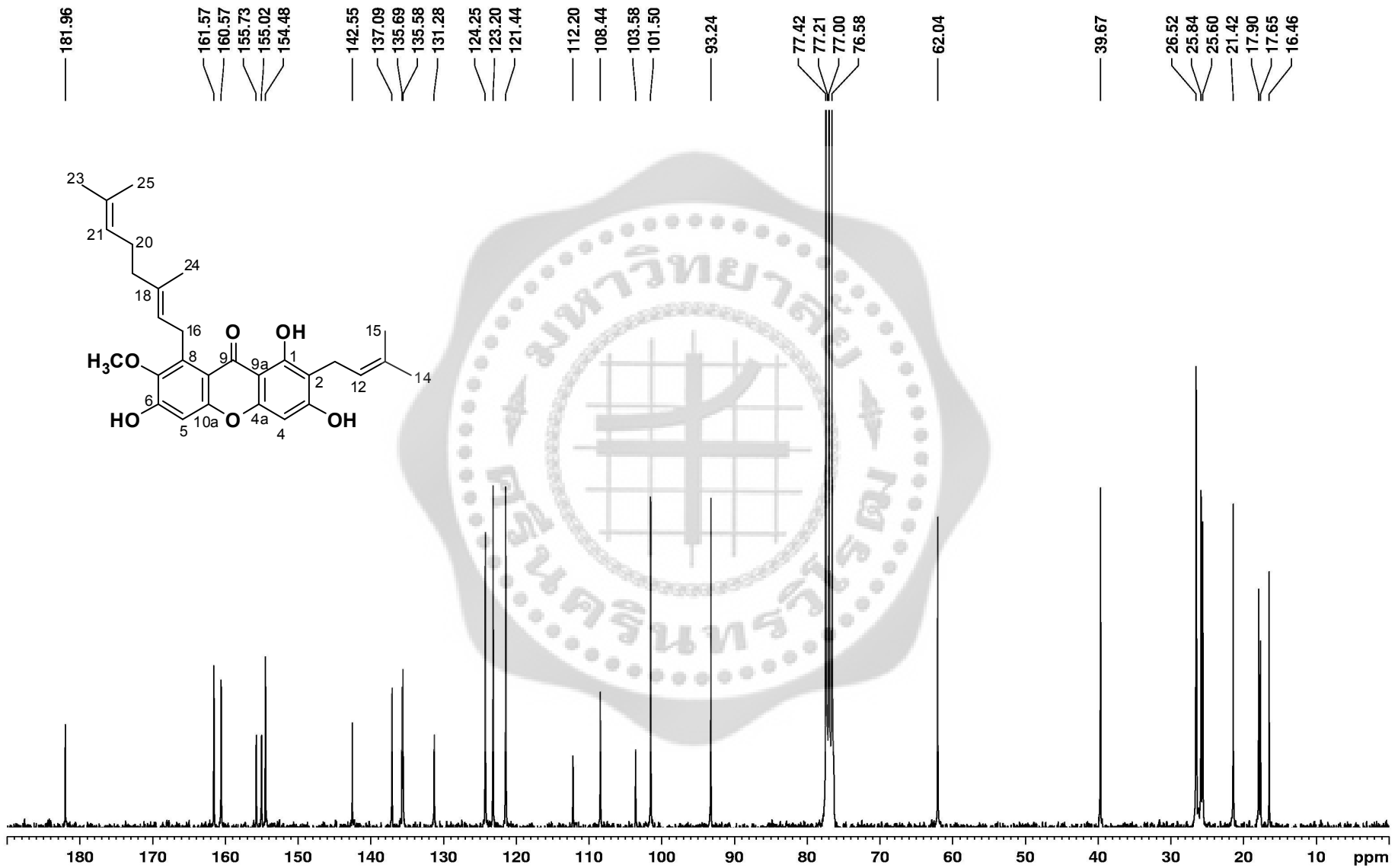

Figure 27 ^{13}C NMR of compound B (cowanin, sss4652) in $CDCl_3$

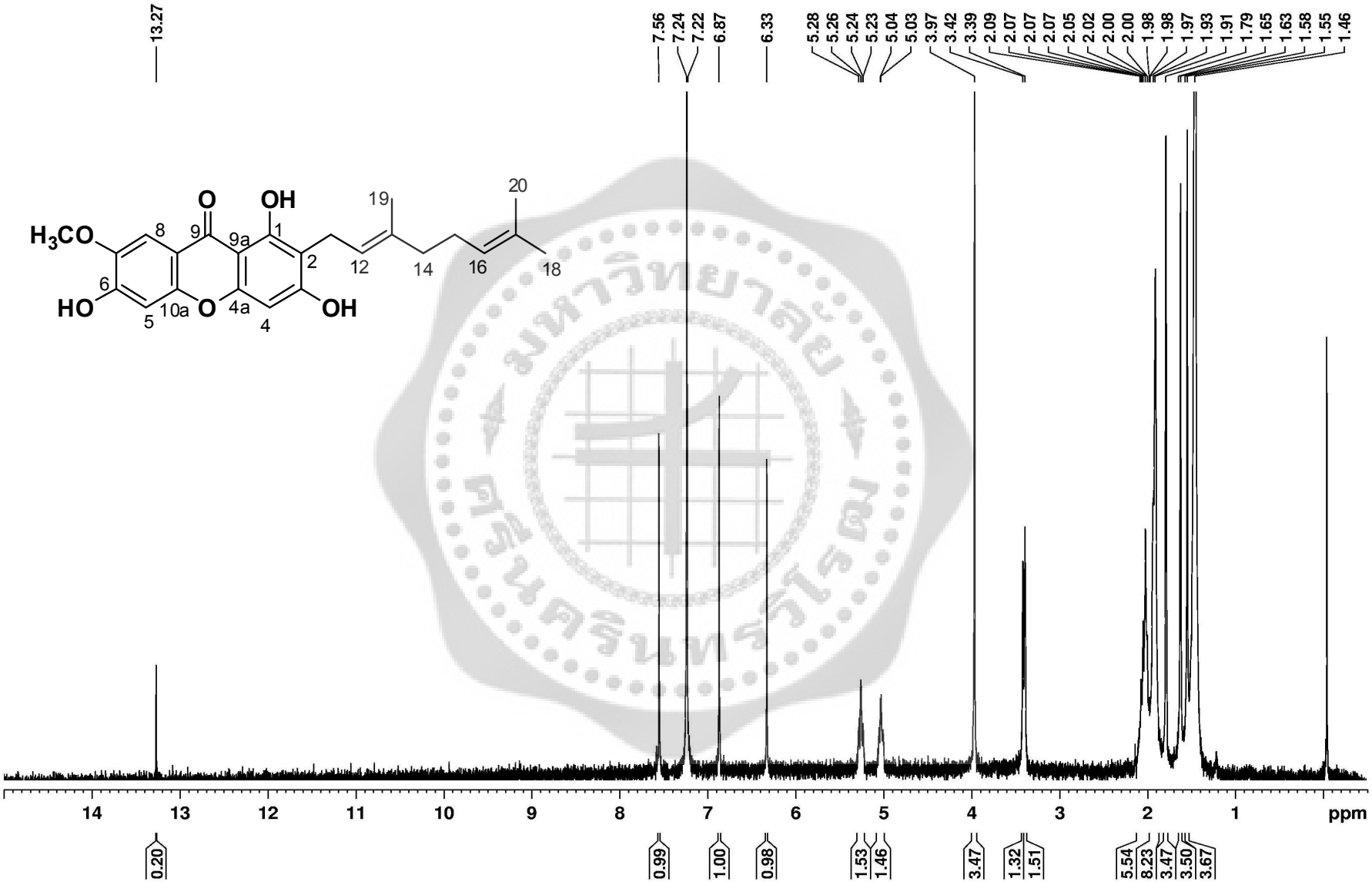

Figure 28 ^{1}H NMR of compound C (cowaxanthone, sss4223) in $CDCl_3$ + MeOD

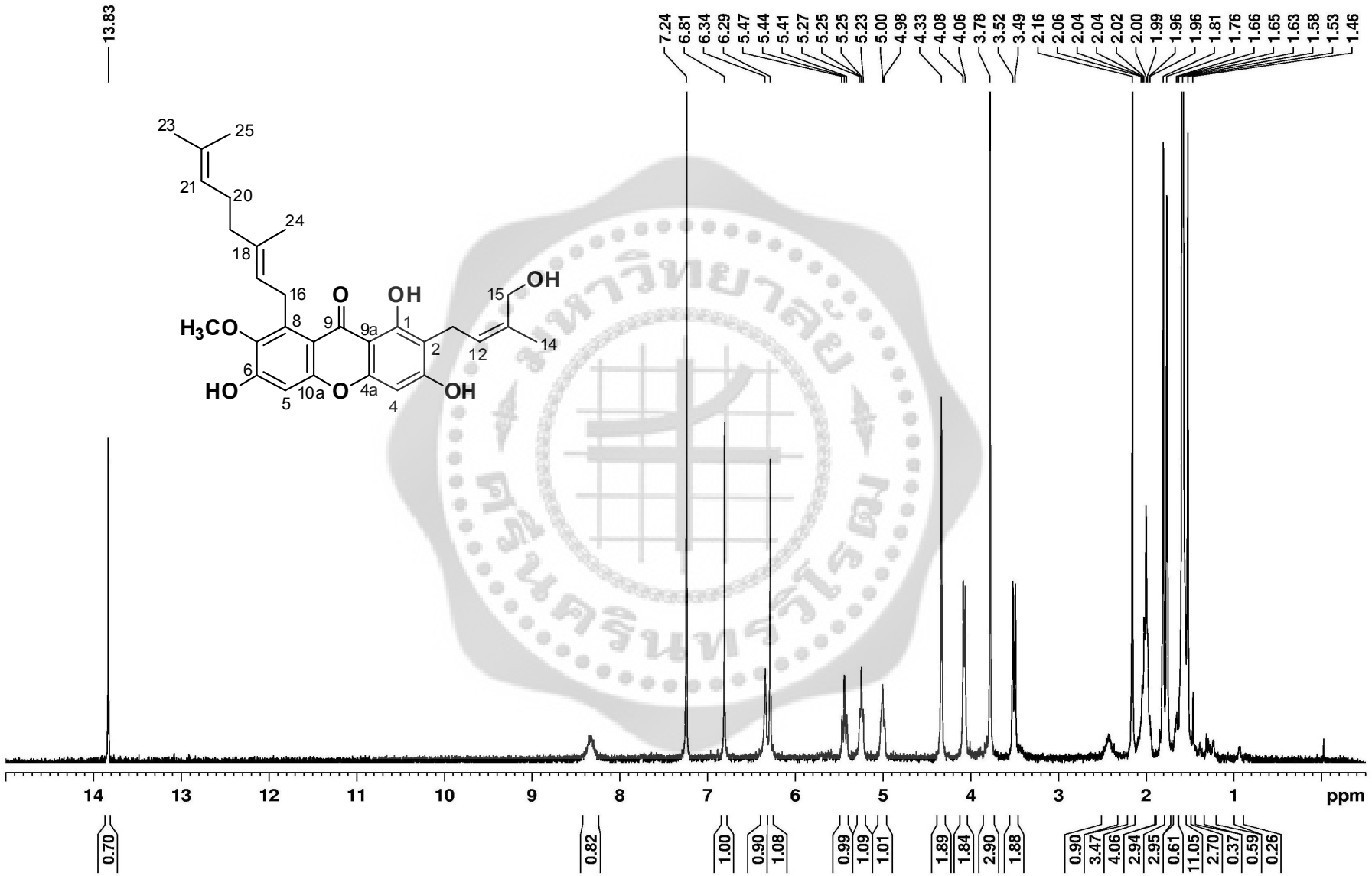

Figure 29 ^{1}H NMR of compound D (cowanol, sss4247) in $CDCl_3$

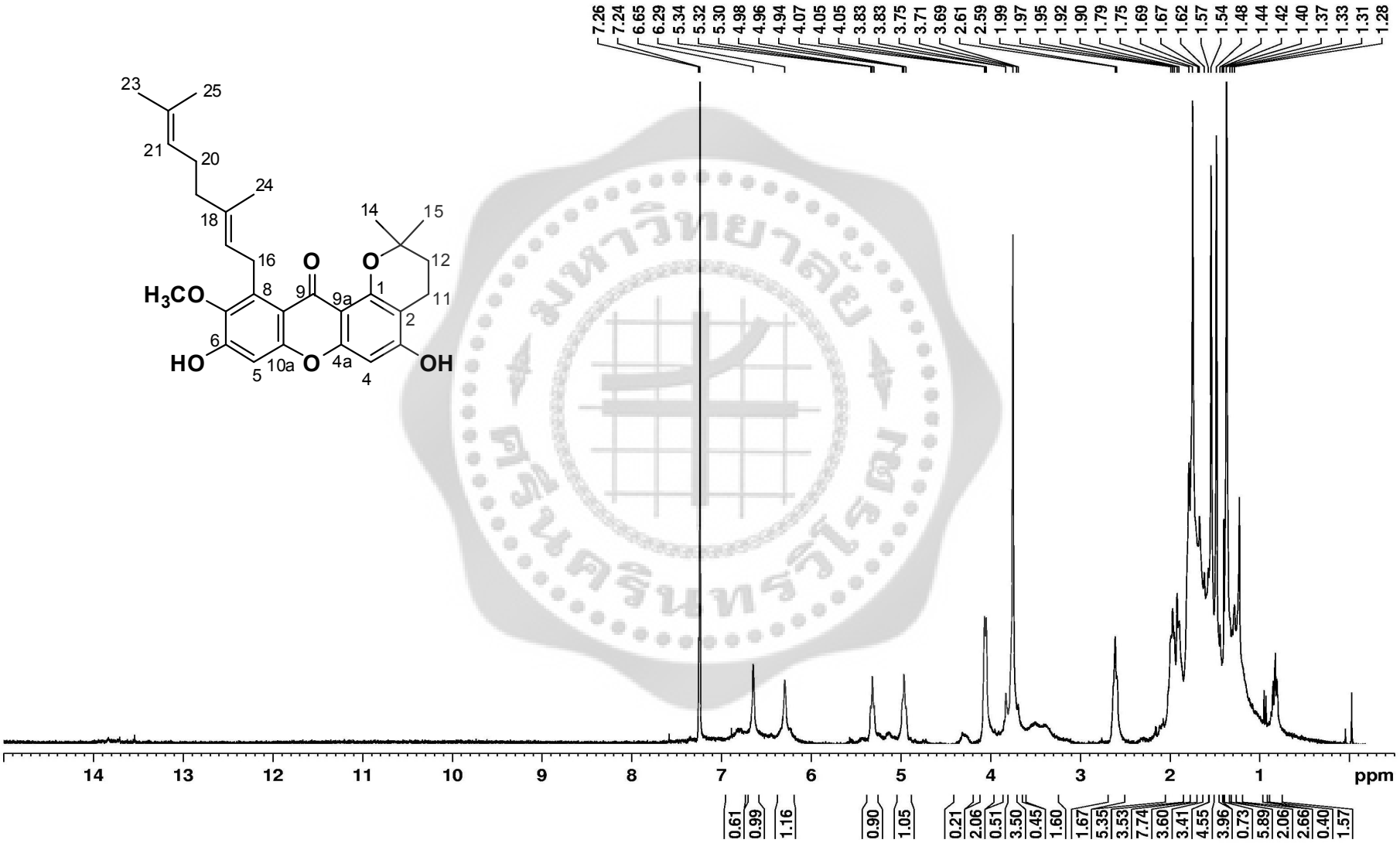

Figure 30 ^{1}H NMR of compound E (fuscaxanthone G, sss4527) in $CDCl_3$

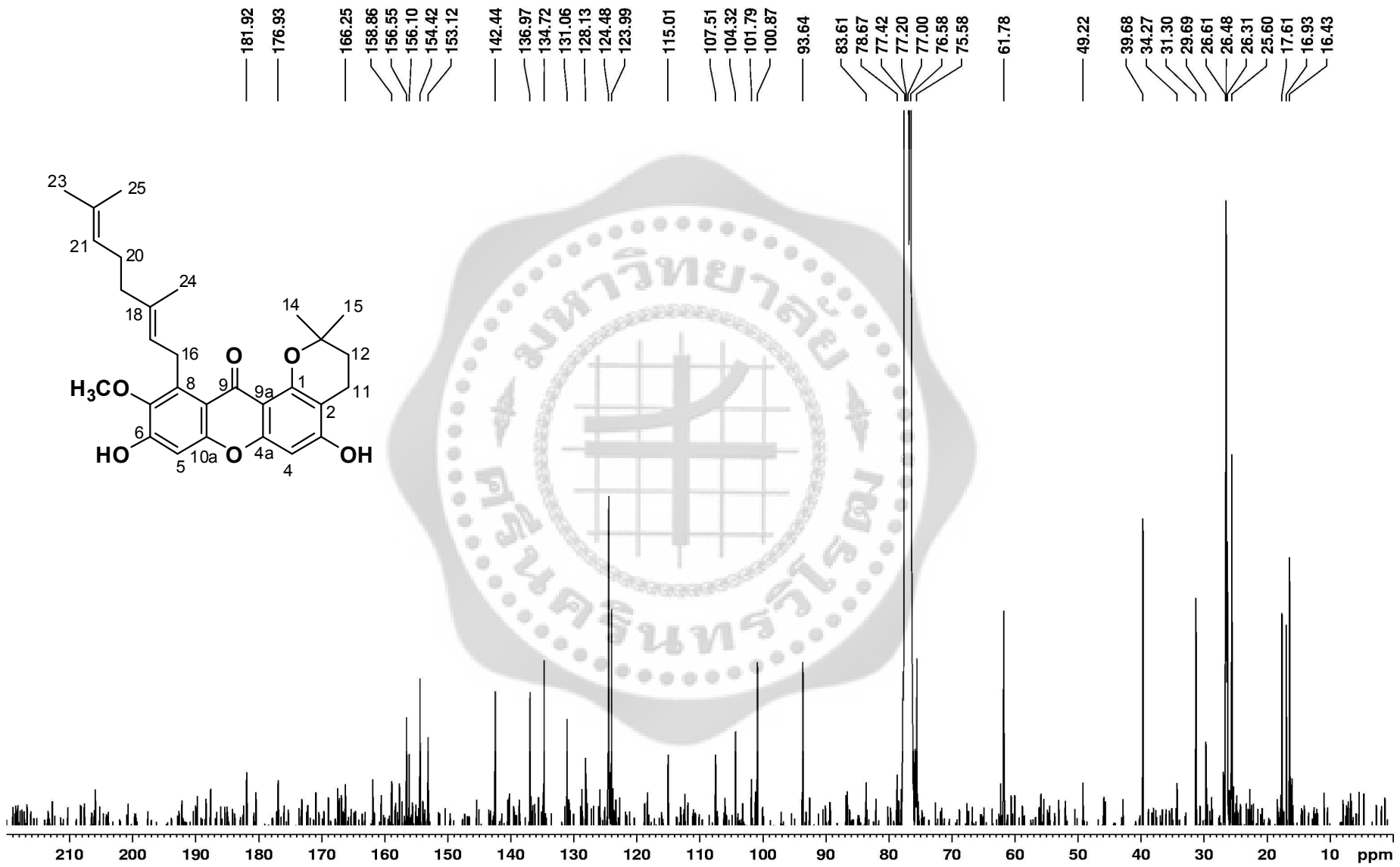

Figure 31 ^{13}C NMR of compound E (fuscaxanthone G, sss4527) in $CDCl_3$

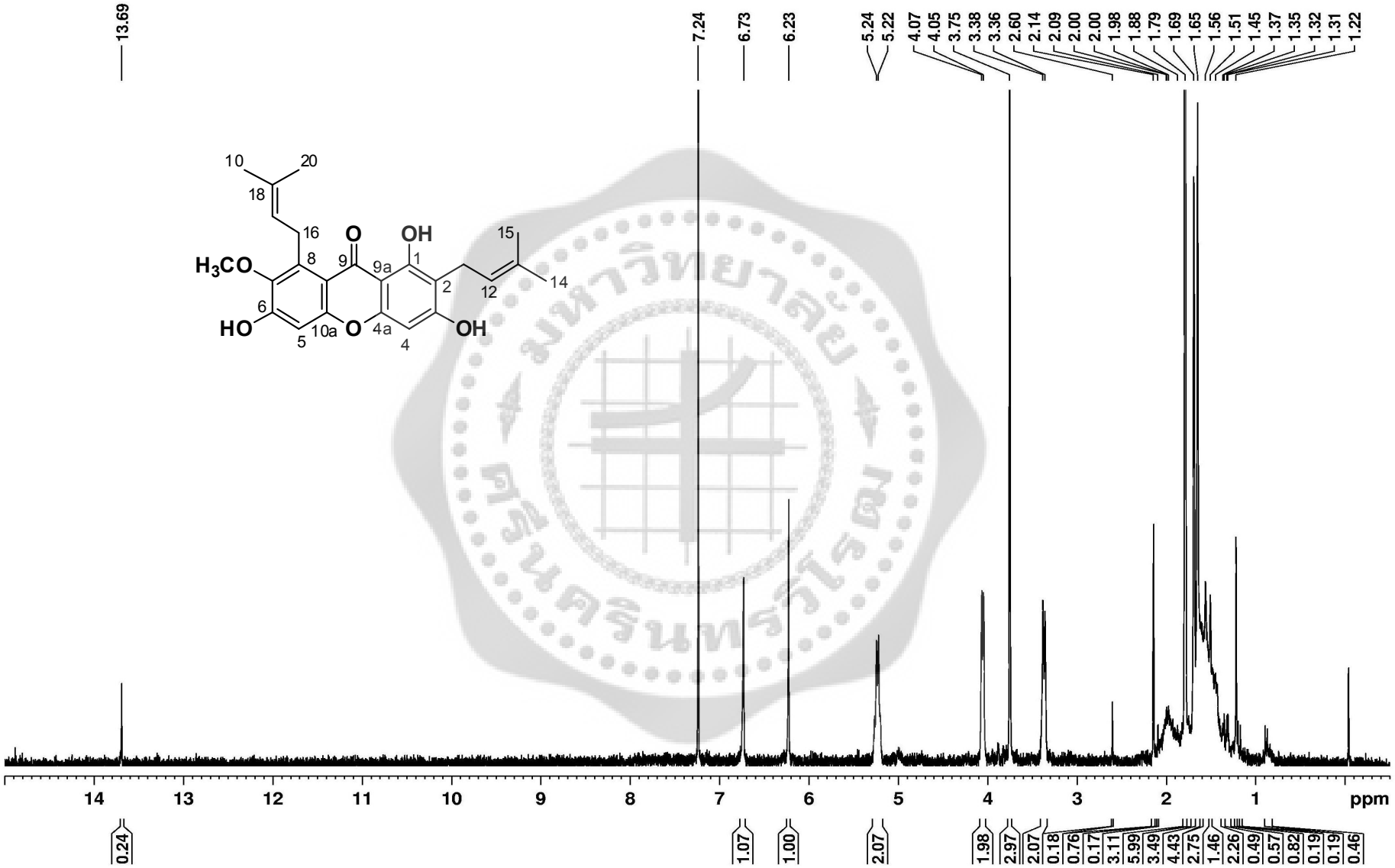

Figure 32 ^{1}H NMR of compound F (α-mangostin, sss4384) in $CDCl_3$ + MeOD

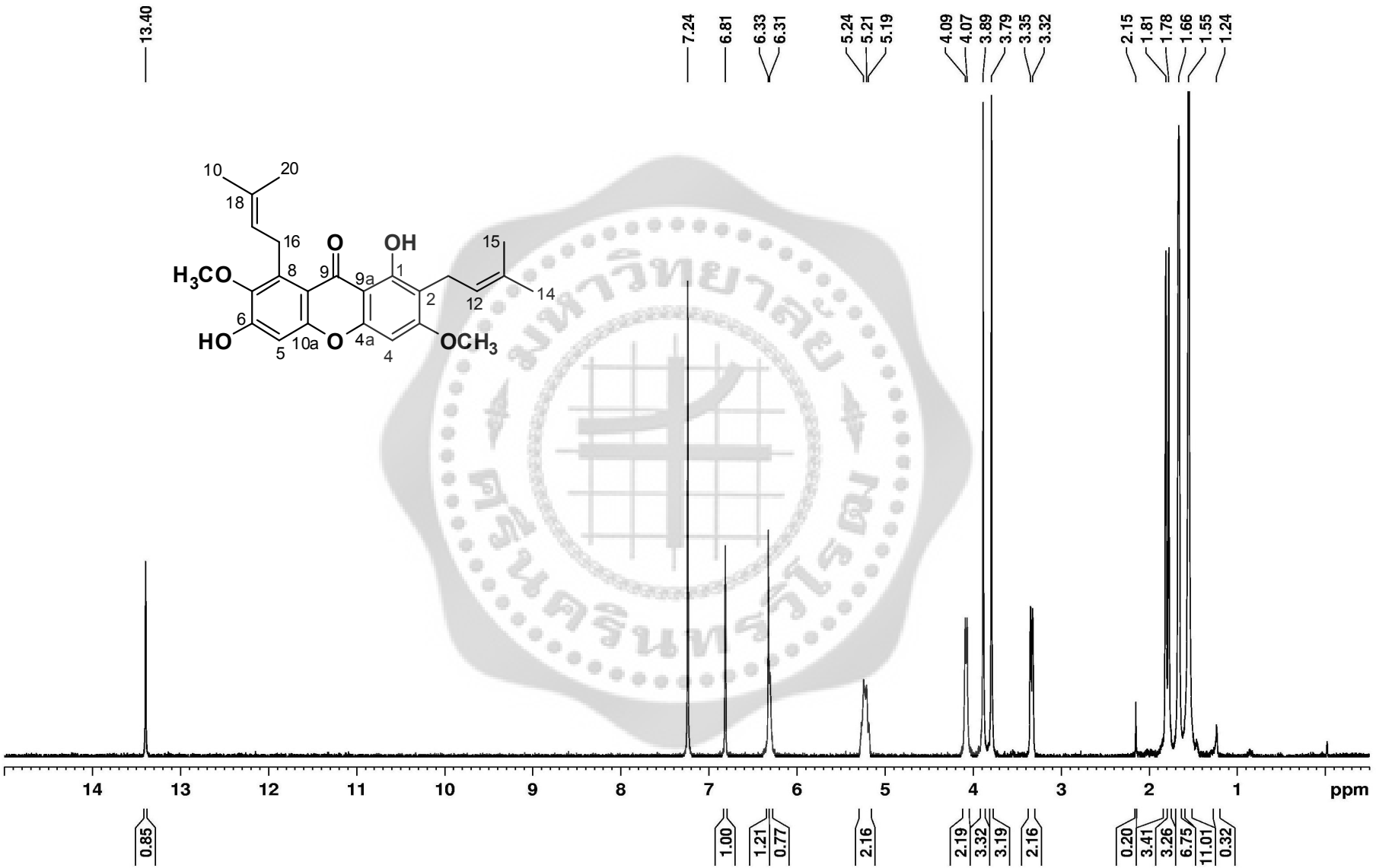

Figure 33 ^{1}H NMR of compound G (β-mangostin, sss4532) in $CDCl_3$

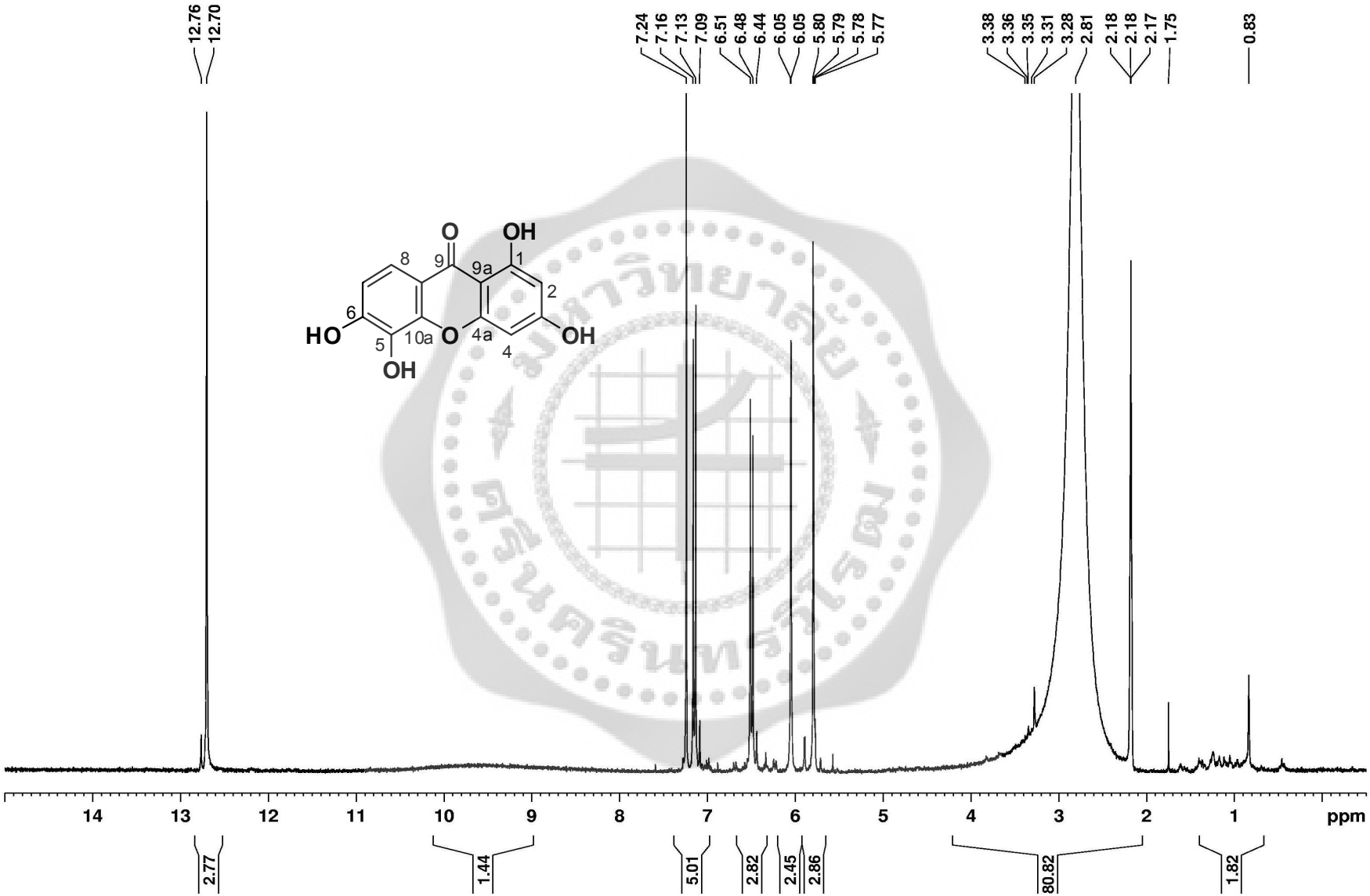

Figure 34 ^{1}H NMR of compound H (1,3,5,6,-tetrahydroxyxanthone, sss4863) in $CDCl_3$ + DMSo-d_6

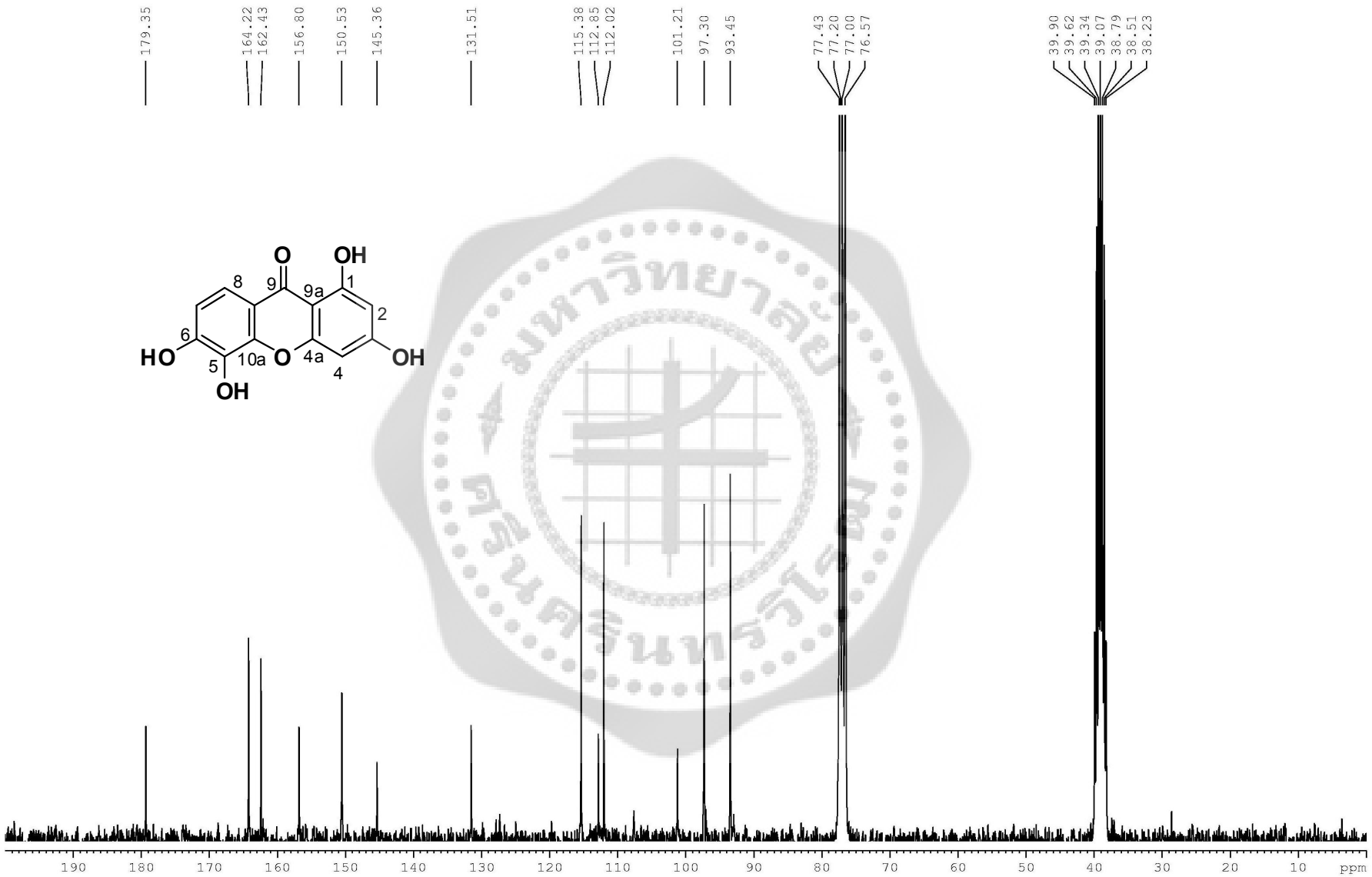

Figure 35 ^{13}C NMR of compound H (1,3,5,6,-tetrahydroxyxanthone, sss4863) in $CDCl_3$ + DMSo-d_6

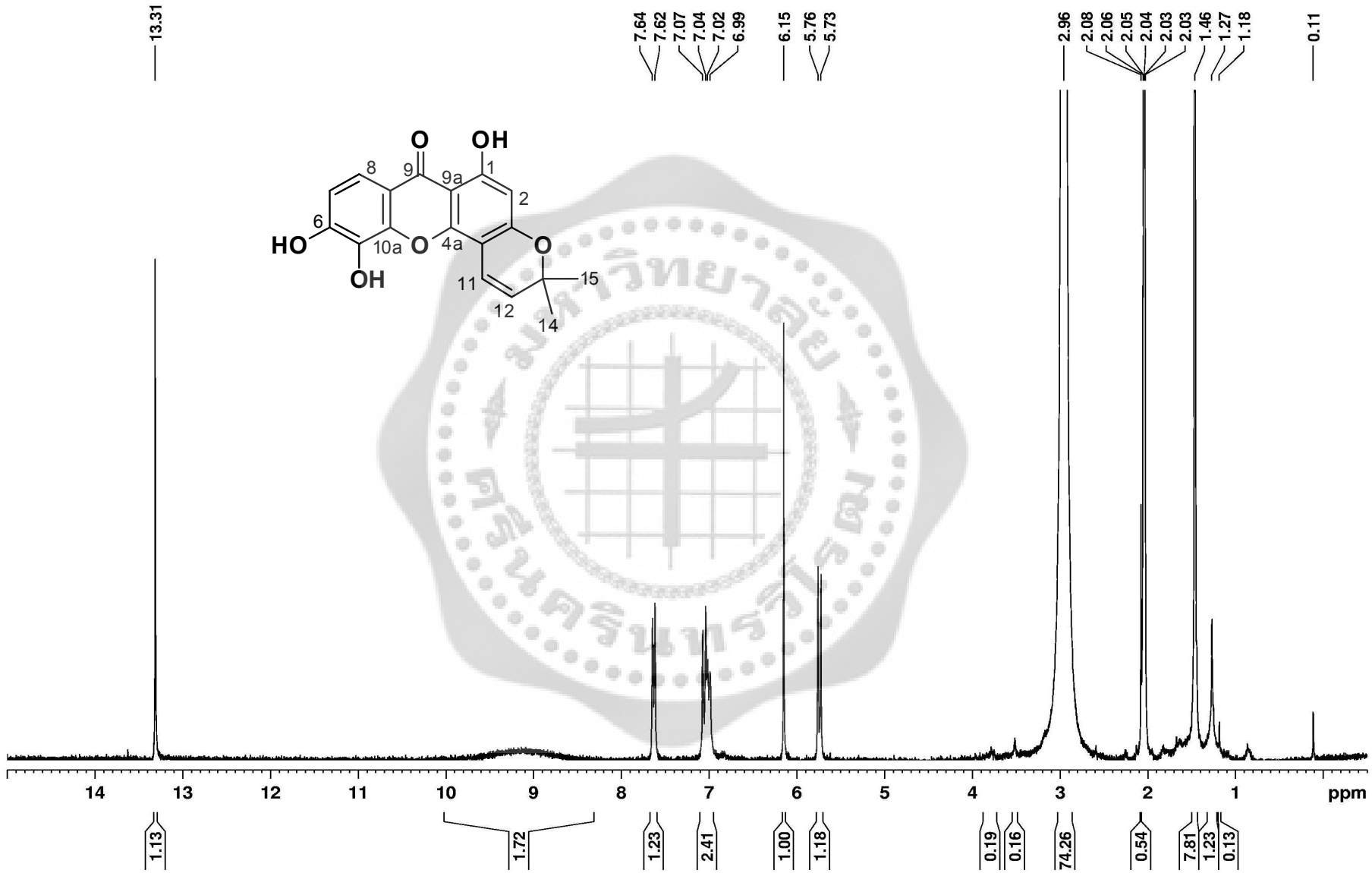

Figure 36 ^{1}H NMR of compound I (isojacareubin, sss4310) in acetone-d_6

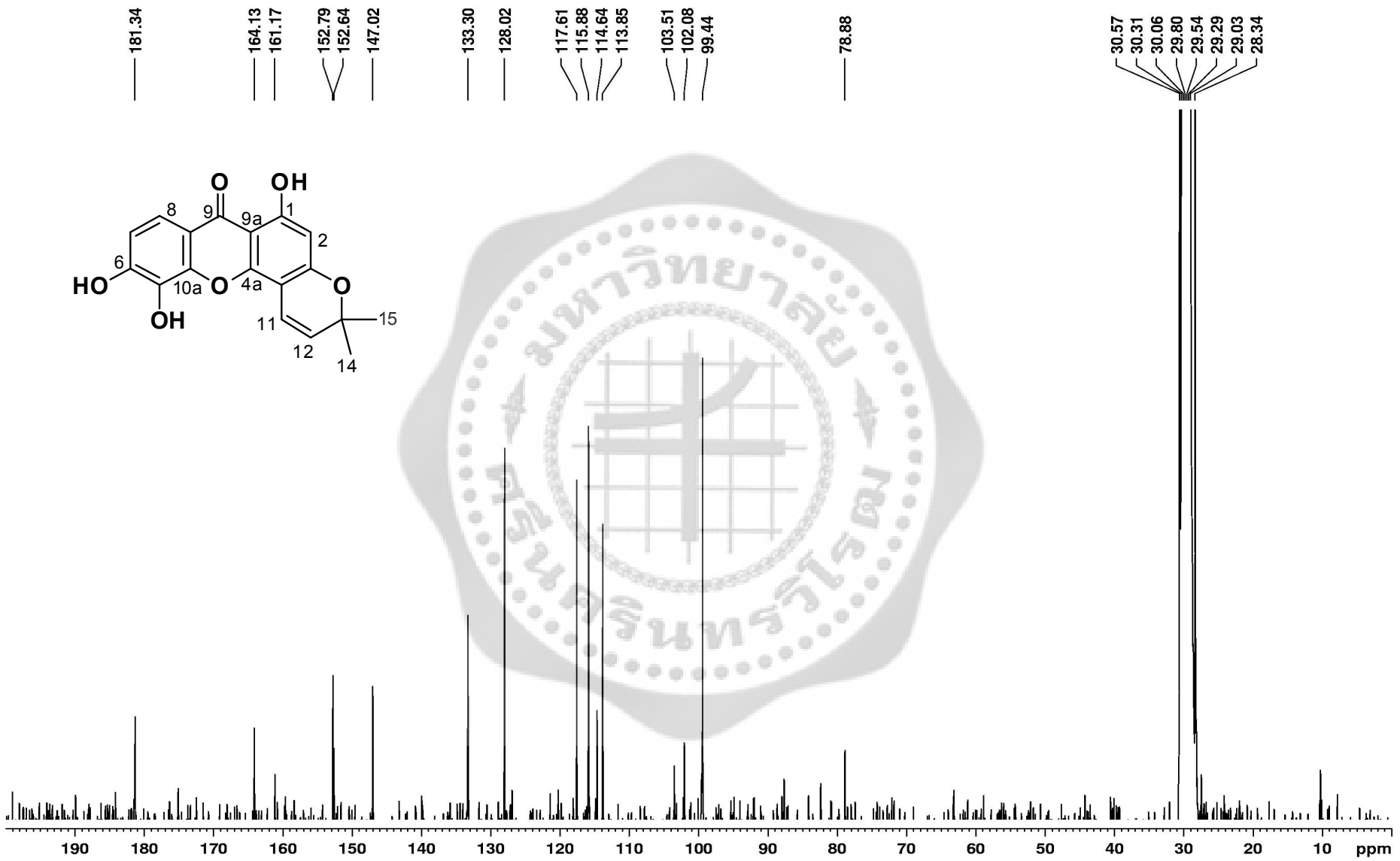

Figure 37 ^{13}C NMR of compound I (isojacareubin, sss4310) in acetone-d_6

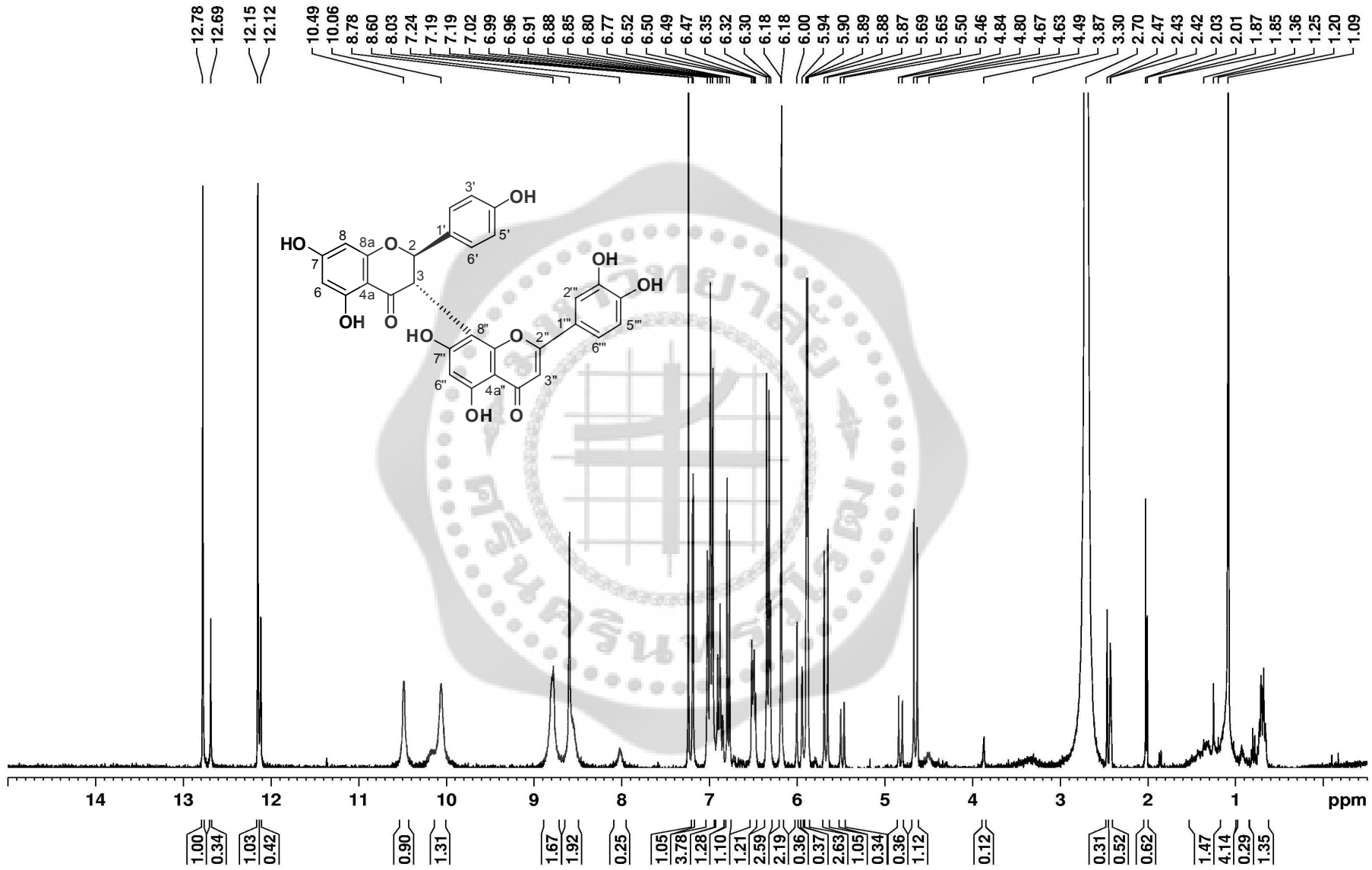

Figure 38 ^{1}H NMR of compound J (morelloflavone, sss4665) in $CDCl_3$ + MeOD

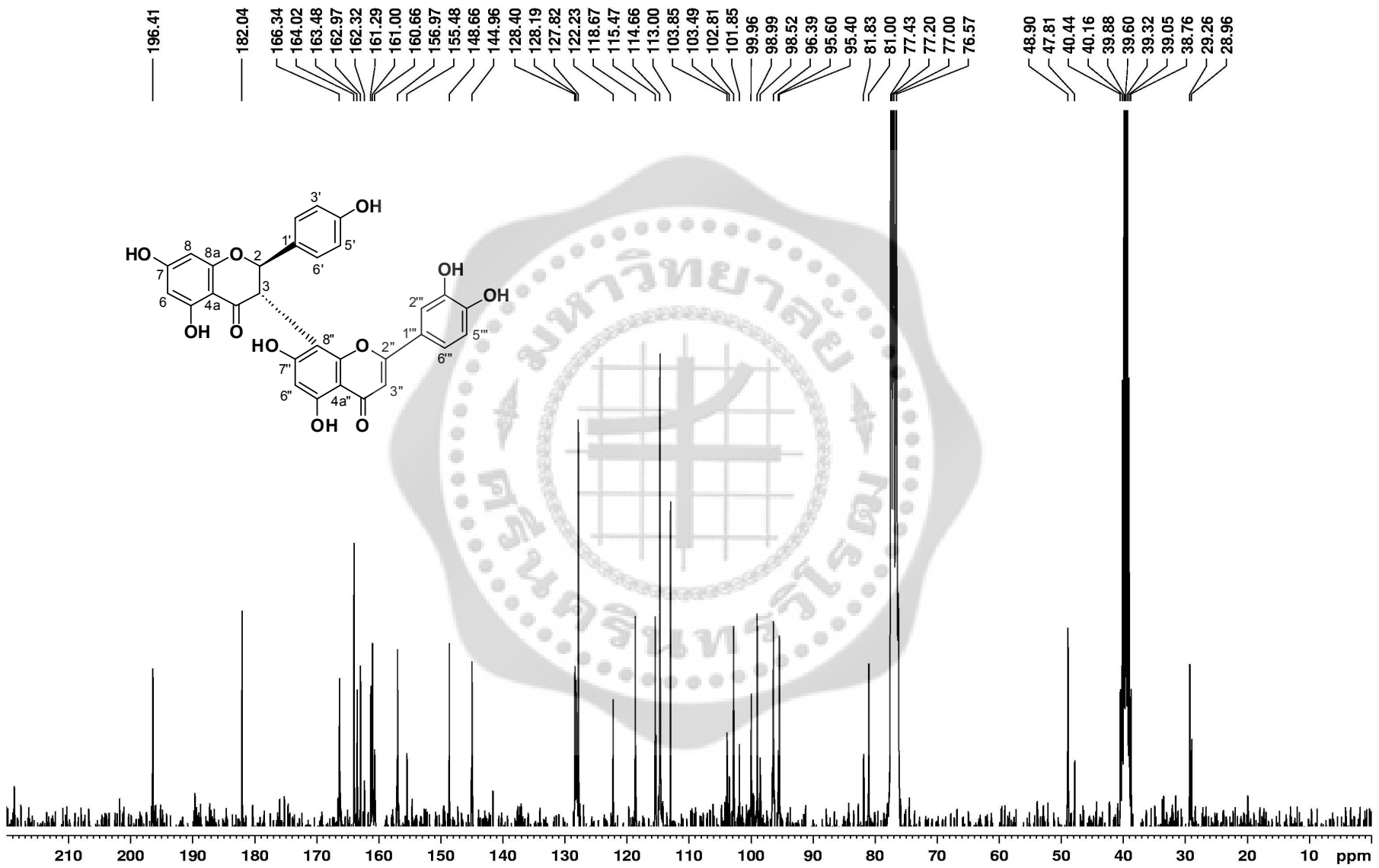

Figure 39 ^{13}C NMR of compound J (morelloflavone, sss4665) in $CDCl_3$ + MeOD

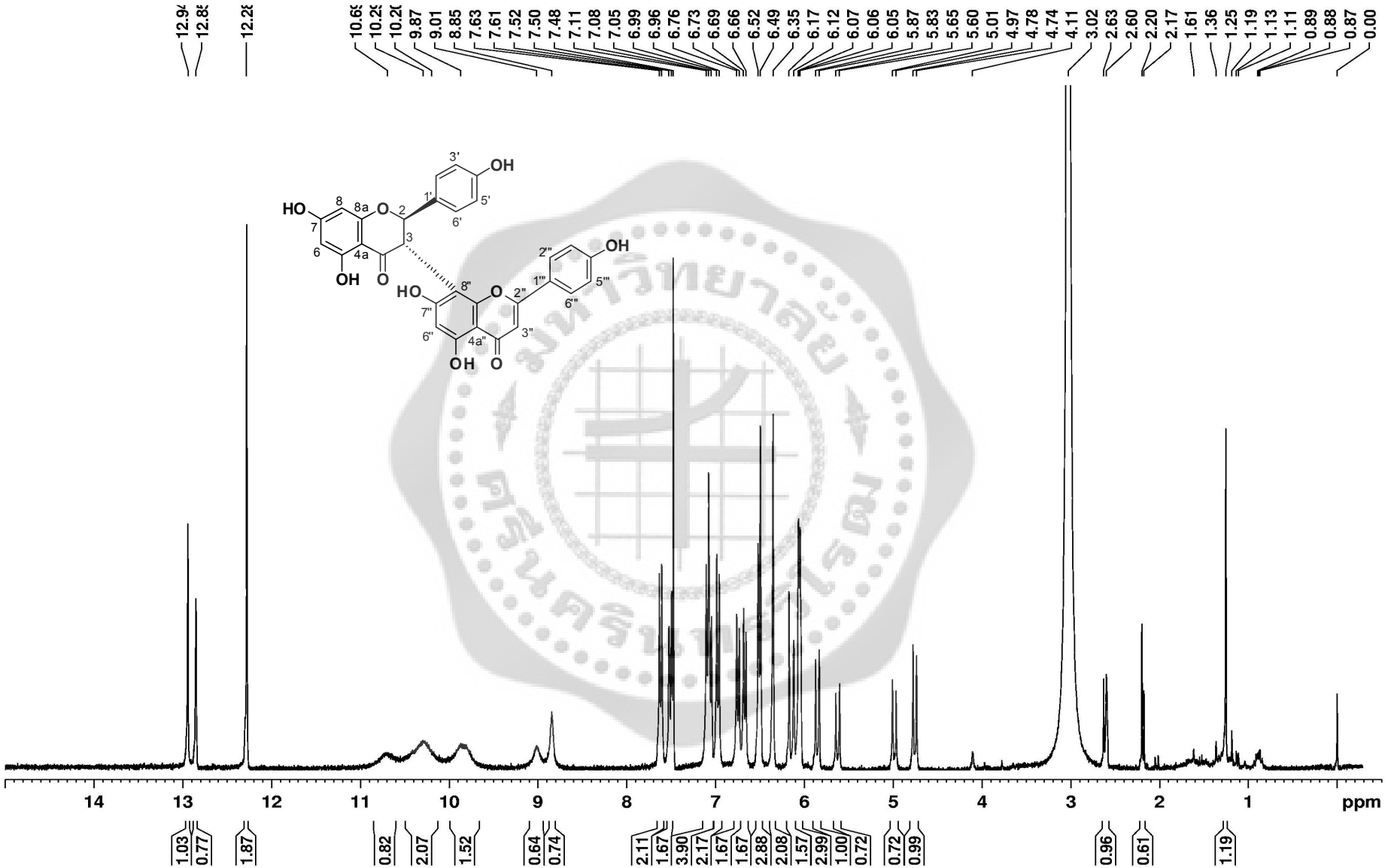

Figure 40 ^{1}H NMR of compound K (vokensiflavone, sss4575) in $CDCl_3$ $CDCl_3$ + MeOD

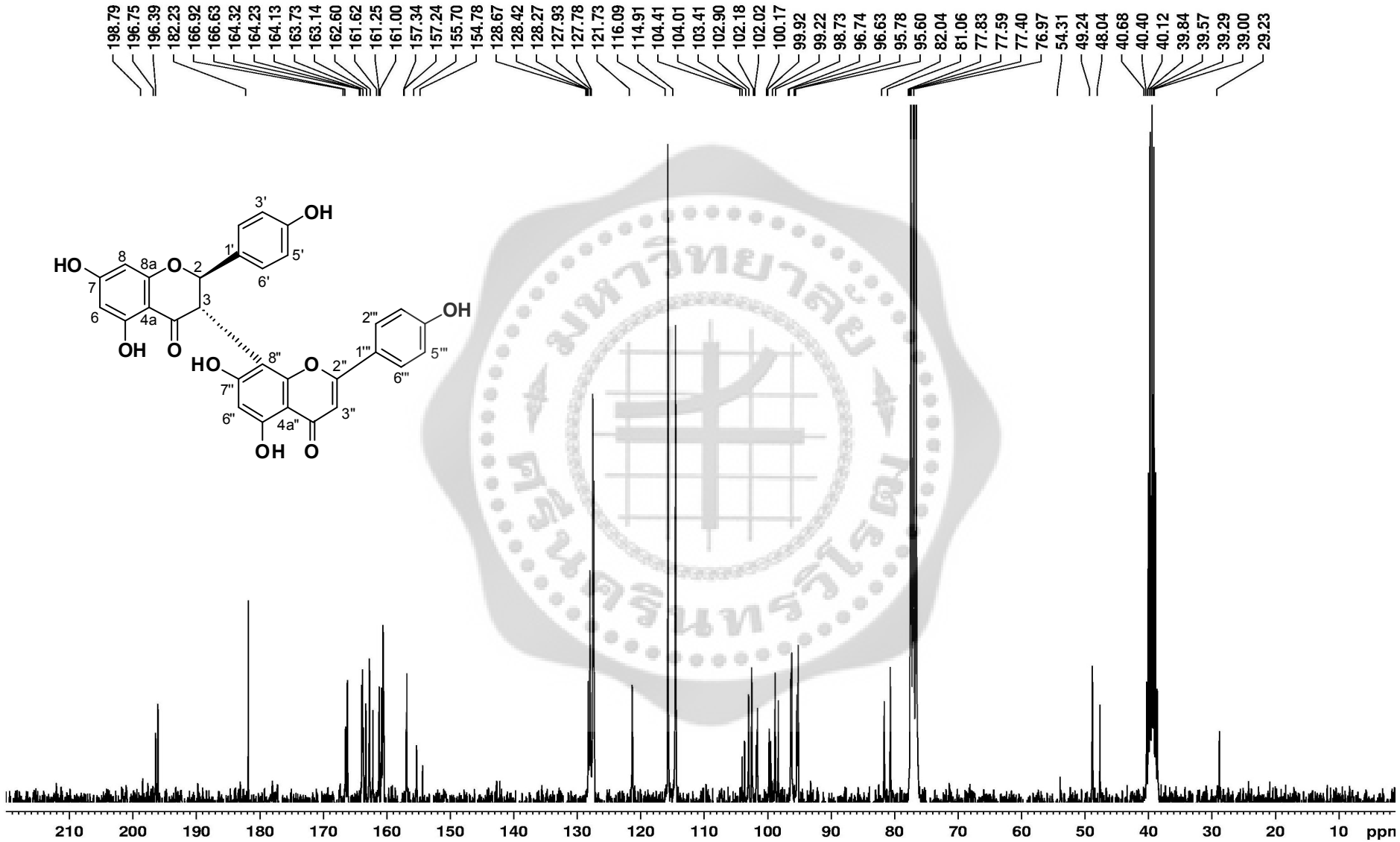

Figure 41 ^{13}C NMR of compound K (vokensiflavone, sss4575) in $CDCl_3$ + MeOD

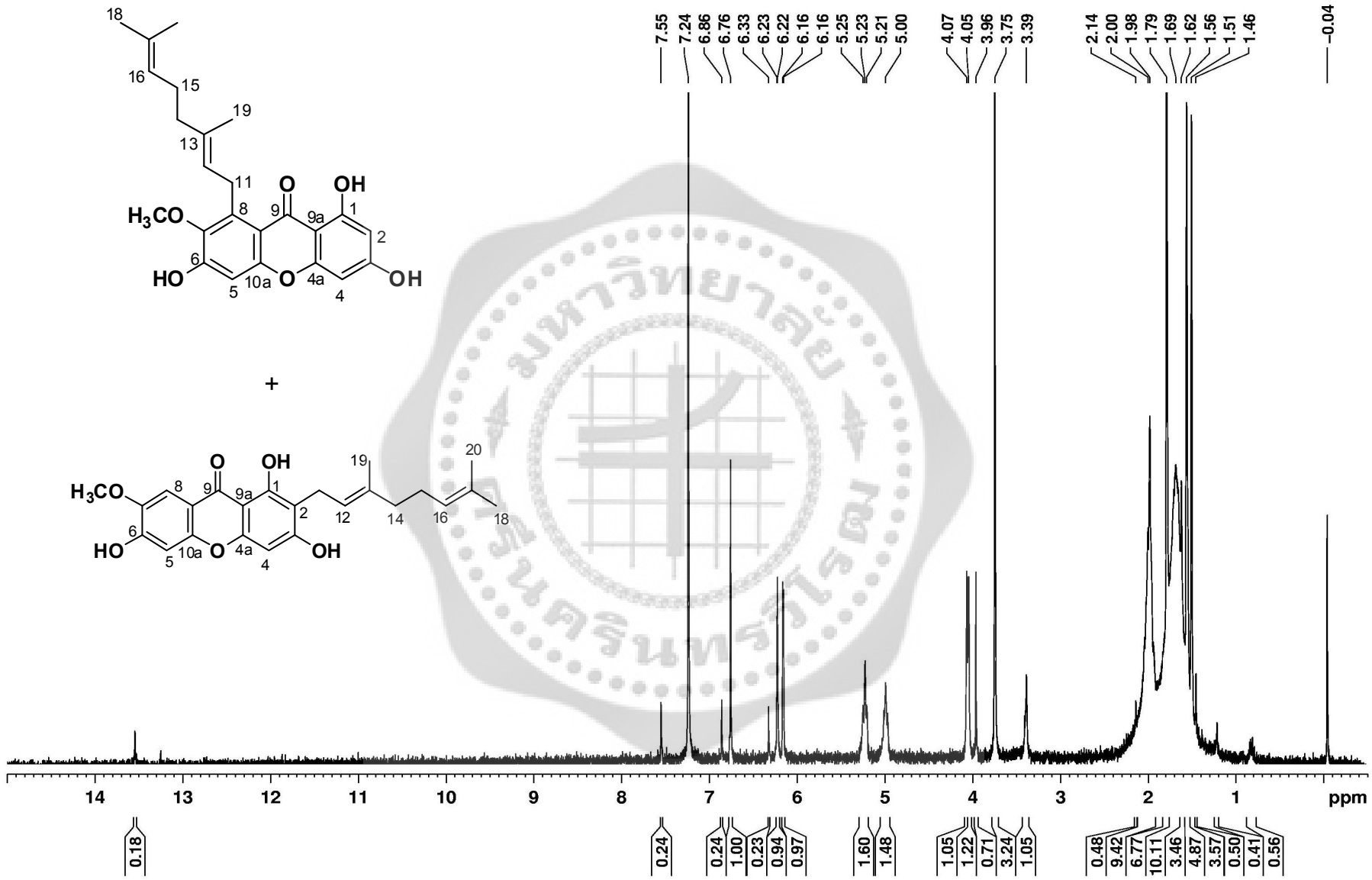

Figure 42 ^{1}H NMR of compound L (rubraxanthone + cowaxanthone, sss4219 in $CDCl_3$

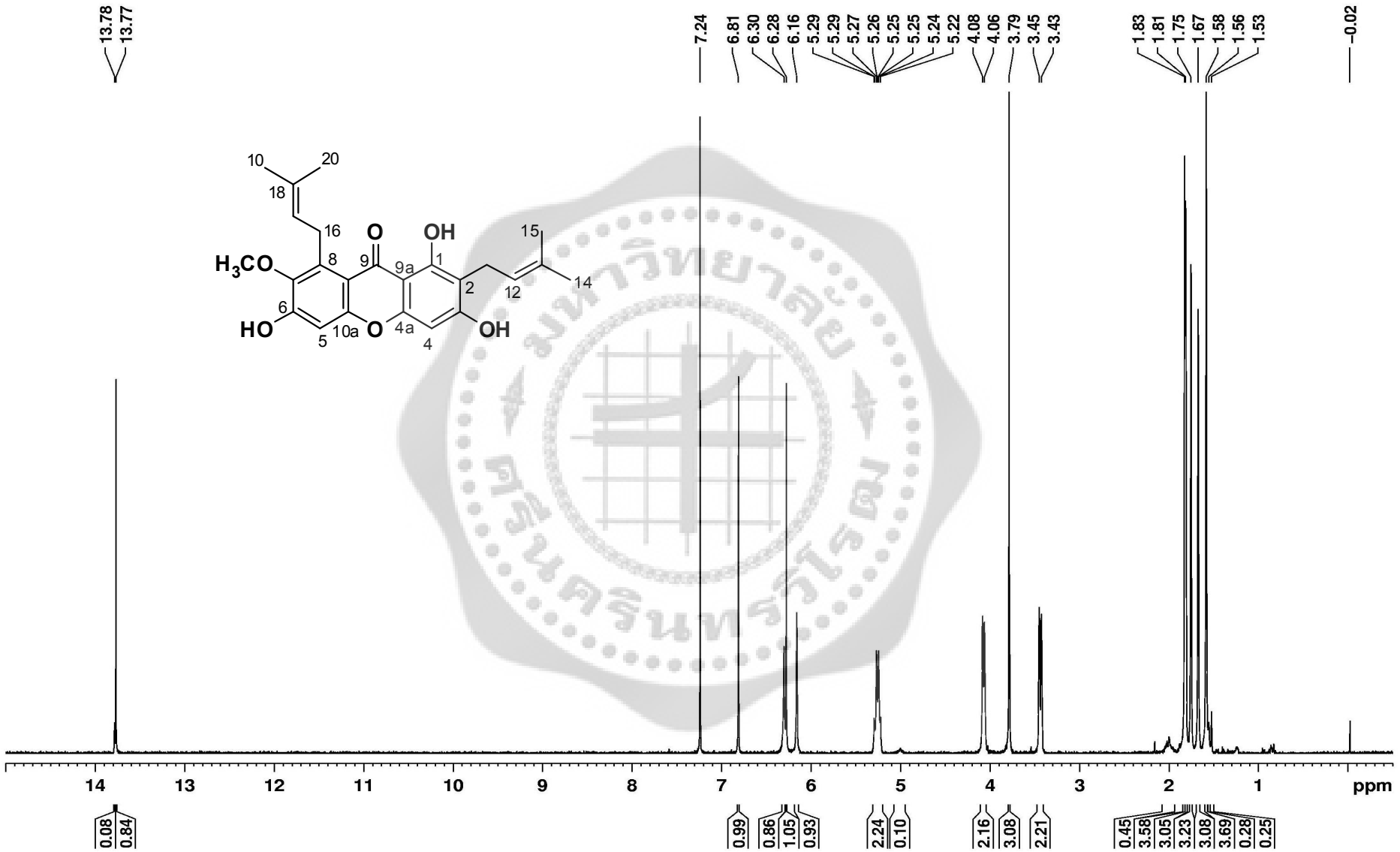

Figure 43 ^{1}H NMR of compound M (α-mangostin, sss4609) in $CDCl_3$

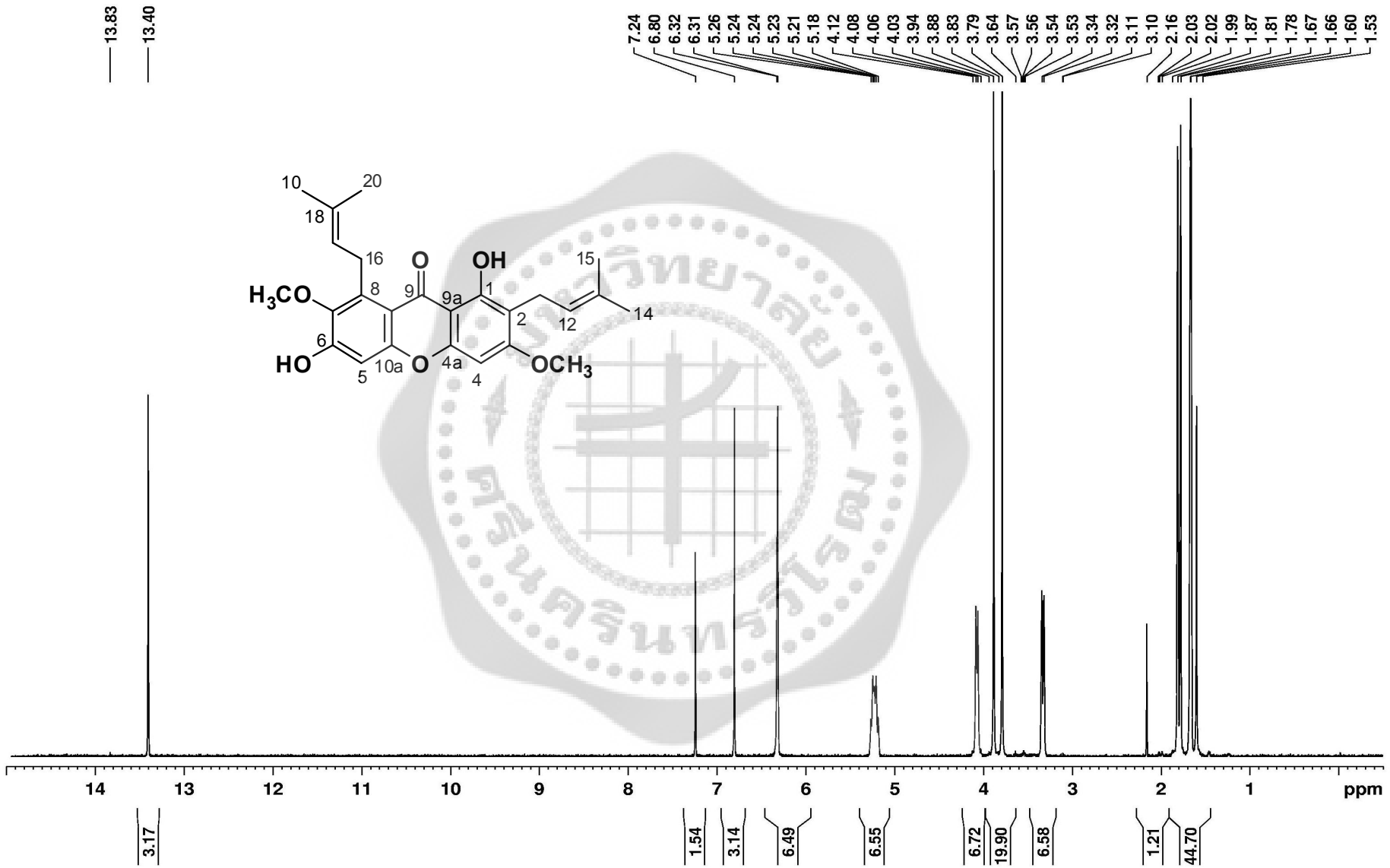

Figure 44 ^{13}C NMR of compound N (β-mangostin, sss4474) in $CDCl_3$

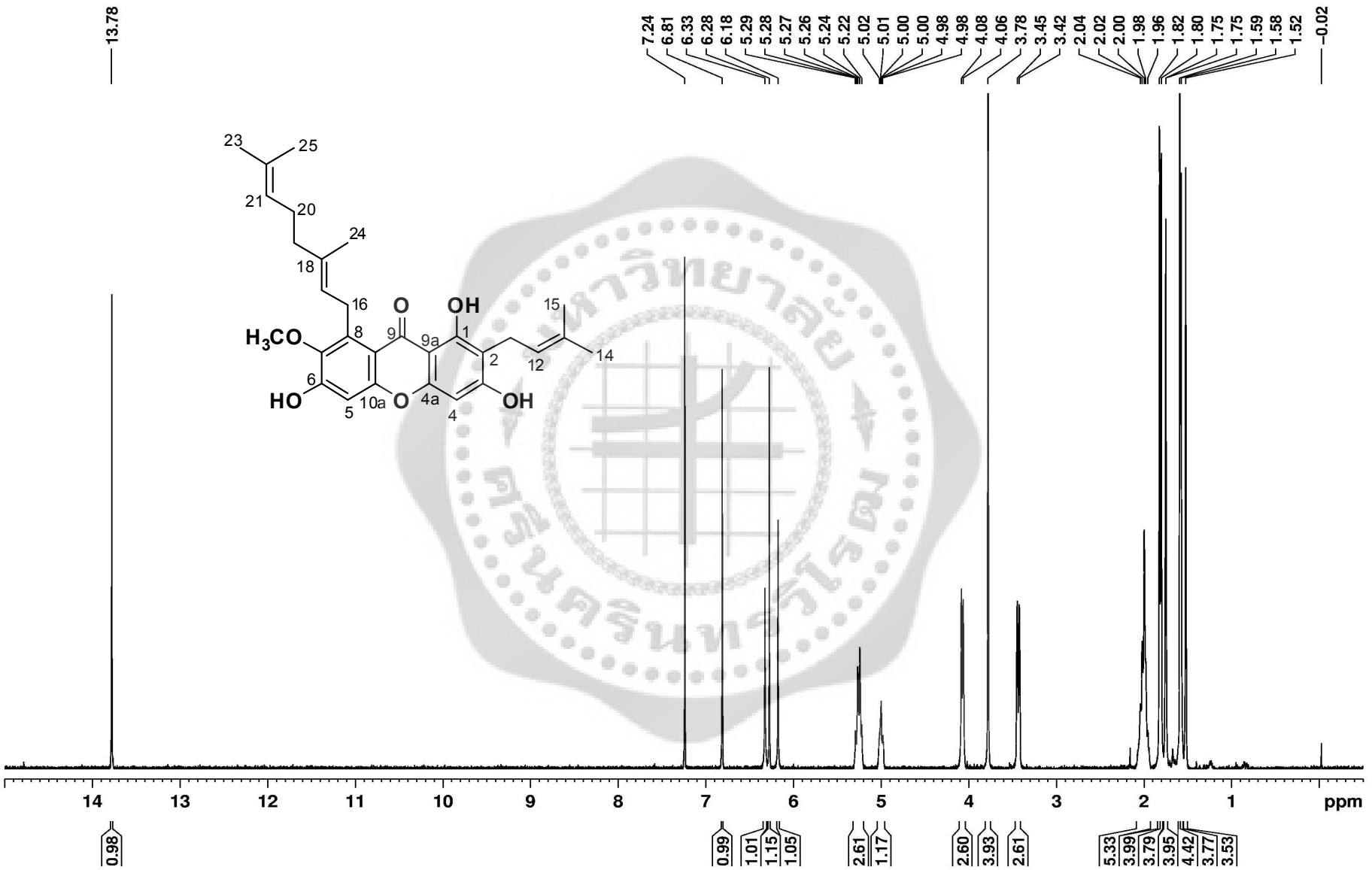

Figure 45 ^{1}H NMR of compound O (cowanin, sss4652) in $CDCl_3$

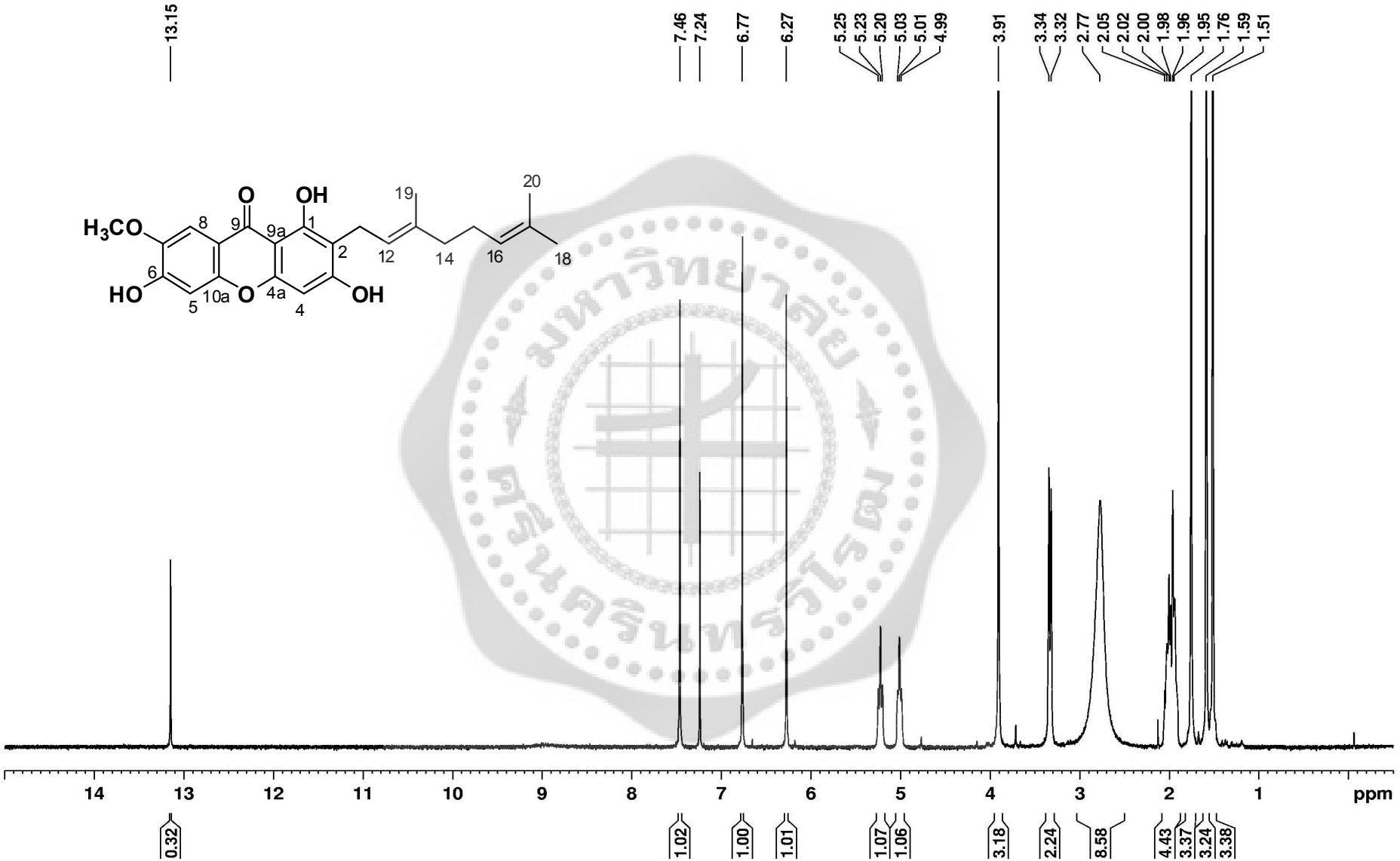

Figure 46 ^{1}H NMR of compound P (cowaxanthone, sss4777) in $CDCl_3$

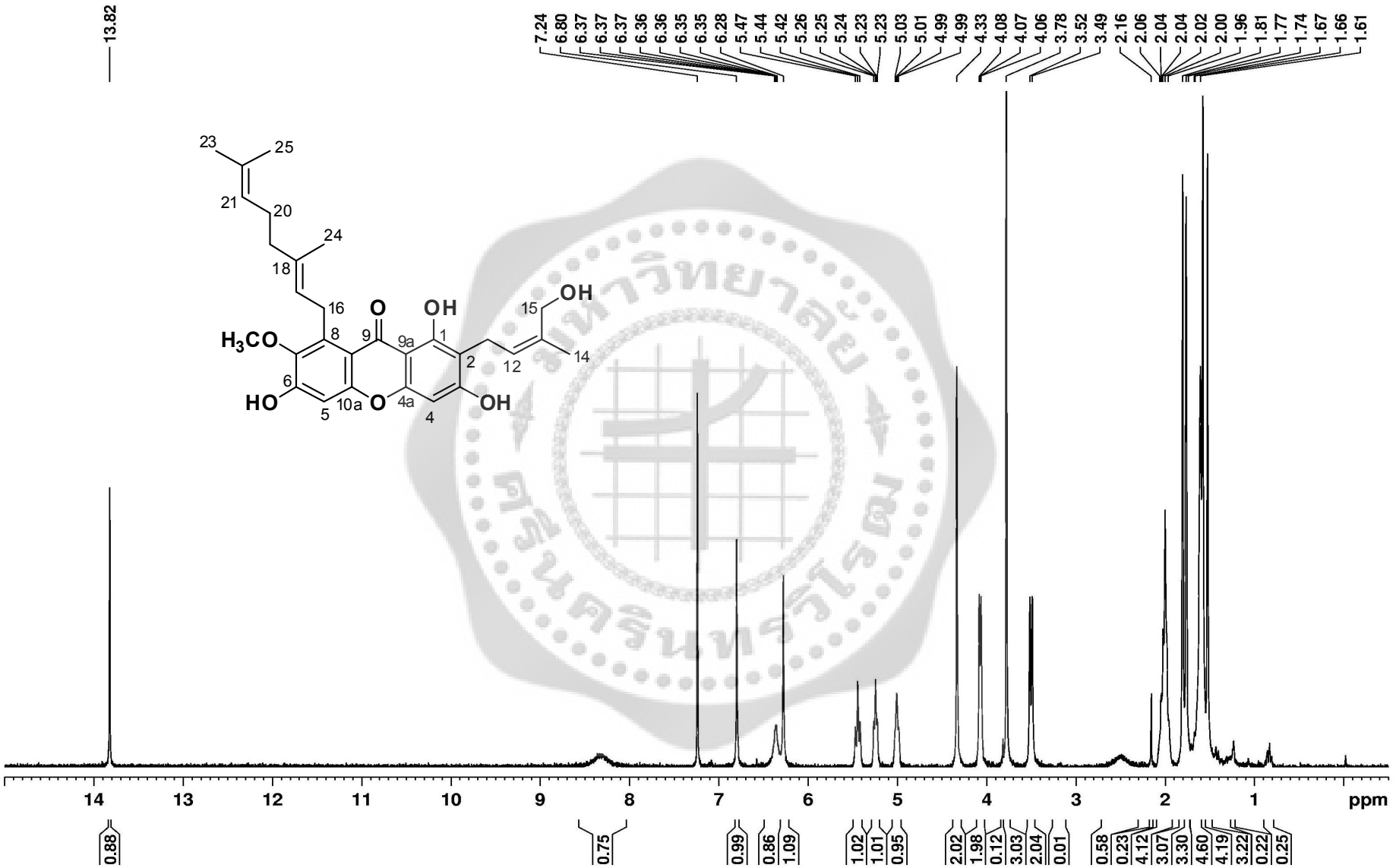

Figure 47 ^{1}H NMR of compound P (cowaxanol, sss4528) in $CDCl_3$

GLOSSARY

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D^{28.5}$

Specific rotation at 28.5° and sodium D line

δ

Chemical shift (for NMR data)

ε

Molar absorptivity

μL

Microliter

μM

Micromolar

λ_{max}

Wavelength at maximal absorption

ν_{max}

Wave number at maximal absorption

$[M+H]^+$

Protonated molecular ion

^{13}C NMR

13-Carbon Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR

Proton Nuclear Magnetic Resonance Spectroscopy

^{1}H-^{1}H COSY

Homonuclear (Proton-Proton) Correlation Spectroscopy

br s

Broad singlet (for NMR data)

br t

Broad triplet (for NMR data)

calcd

Calculated

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

CC

Column chromatography

$CDCl_3$

Deuterated chloroform

CH_2Cl_2

Dichloromethane

$CHCl_3$

Chloroform

cm

Centimeter

cm^{-1}

Reciprocal centimeter (unit of wave number)

d

Doublet (for NMR data)

dd

Doublet of doublets (for NMR data)

ddd

Double doublet of doublets (for NMR data)

DEPT

Distortionless Enhancement by Polarization Transfer

EIMS

Electron-Ionization Mass Spectrometry

ESIMS

Electrospray ionization Mass Spectrometry

EtOAc

Ethyl acetate

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

g

Gram

Glc

Glucoside

h

Hour

H_2O

Water

HMBC

^{1}H-Detected Heteronuclear Multiple Bond Coherence

HMQC

^{1}H-Detected Heteronuclear Multiple Quantum Coherence

Hz

Hertz

IC_{50}

50% Inhibitory Concentration

IR

Infrared

J

Coupling constant

KBr

Potassium bromide

kg

Kilogram

L

Liter

m

Multiplet (for NMR data)

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

mg

Milligram

MIC

Minimum Inhibitory Concentration

mL

Milliliter

mm

Millimeter

NMR

Nuclear Magnetic Resonance Spectroscopy

NOESY

Nuclear Overhauser Effect Spectroscopy

°C

Degree Celsius

QCC

Quick column chromatography

s

Singlet (for NMR data)

t

Triplet (for NMR data)

TLC

Thin Layer Chromatography

UV

Ultraviolet

α

Alpha

β

Beta

CURRICULUM VITAE

CURRICULUM VITAE

Name : Jannarin Nontakham

Date of Birth : August 26, 1985

Place of Birth : KhonKaen

Address : 31/13 Tumbol Buayai, Nampong District, KhonKaen, Thailand

Educational Background:

2008 Bachelor of Science Degree in Chemistry
Srinakharinwirot University, Bangkok, Thailand

2011 Master of Science Degree in Chemistry
Srinakharinwirot University, Bangkok, Thailand

Scholarships:

2008-2011 Research assistantship

2009-2010 The Center of Excellence for Innovation in Chemistry (PERCH-CIC)

2011 The Strategic Basic Research Grant of The Thailand Research Fund.

Proceeding:

Nontakham, J.; Ruamsanith, D.; Puangjan, A.; Komutiban, O.; Suksamrarn, S. Xanthones from the Fresh Green Fruit of *Garcinia fusca* Pierre. 5th SWU Conference, Srinakharinwirot University, Bangkok, Thailand, March 17-18, 2011. Presentation number SWU5-1023.

Poster presentation:

Nontakham, J.; Raveevan Jittopas.; Ukkarapong Krompo.; Ruamsanith, D.; Suksamrarn, S. Xanthones from the Root of *Garcinia fusca* Pierre. International Congress for Innovation in Chemistry (PERCH-CIC Congress VII), Jomtien Palm Beach Hotel & Resort, Thailand, May 4-7, 2011. Presentation number S2-P26.