Bioactive Compounds from the Seeds of *Mammea siamensis*

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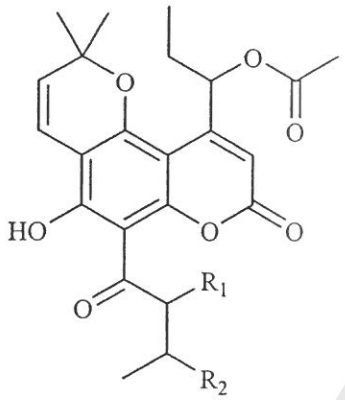
PREFACE

Plants have been used worldwide in traditional medicines for the treatment of diseases. It is estimated that even today approximately two-thirds to three-quarters of the world's population rely only on medicinal plants as their primary source of medicines. In Thailand, several plants have been used by the local Thai people in folk medicine for the treatment of several diseases. For example, some plants in the genus of *Cratoxylum* and *Bruguiera* have been used for the treatment of diarrhea and the healing of wounds. According to many plants have been used for traditional medicines, therefore, the study of phytochemistry and biological activities are very important because the information from the study of bioactive compounds will be used for development and apply into related fields, for example cosmetics, food additives, agricultures and pharmacy. Finally, we hope that the information from our study (bioactives compounds from the seeds *Mammea siamensis*) might be helpful for other researchers who need to study on bioactive compounds and other related fields.

ABSTRACT

The investigation of dichloromethane extract of the seeds of *Mammea siamensis* led to the isolation and identification of five new phenolic compounds (**MS-1-5**). Two of them are new compounds, mammea E/BB cyclo D (**MS-1**) and siamensone A (**MS-5**). All isolates were characterized using 1D and 2D NMR spectral data. In addition, all compounds were evaluated for cytotoxic activity against breast adenocarcinoma (MCF-7), human cervical cancer (HeLa), colon cancer (HT-29) and human oral cancer (KB). In this study, only two compounds (**MS-3** and **4**) were found to be active with IC_{50} value ranging from 0.78-4.64 μg/mL.



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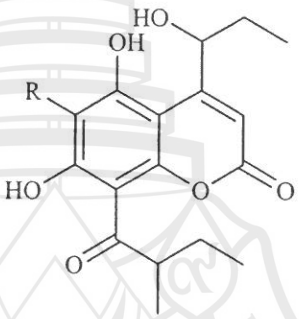

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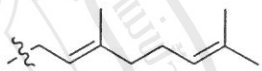

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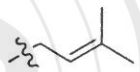

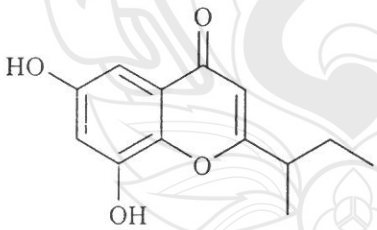

MS-5

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ABBREVIATIONS AND SYMBOLS

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

MS	=	Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
COSY	=	Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
ROESY	=	Rotating from Overhause Effect Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
TMS	=	tetramethylsilane
$CDCl_3$	=	deuterochlroform

CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

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

1.2 Objectives

The objectives of this project are involved:

1. To test biological activity of the crude extracts
2. To isolate and characterize compounds from the crude extracts, which were gave the positive with biological activity testing
3. To test biological activity of pure compounds

1.3 Scope of study

1. Extraction and isolation of secondary metabolite from the seeds of *M. siamensis*

2. Characterization of all isolates by spectroscopic methods, including UV, IR, NMR and MS.

1.4 Benefit

1. Some compounds, which were isolated from the seeds of *M. siamensis*, might be showed significant biological activity.

2. Some active compounds might be applied into the related field i.e. pharmacy, cosmetics and agriculture.

3. Acquire basic knowledge of chemical compounds and biological activity.

4. This work might be published in international journals.

1.5 Literature Reviews

Mammea siamensis (Miq) T. Anders. (Guttiferae), known in Thai as "Sarapi", is a small evergreen tree. The plant was previously known as *Ochrocarpus siamensis* distributed in Thailand, Laos, Cambodia, Vietnam and Myanmar. Two species are found in Thailand (Smitinand, 2001), which are *M. siamensis* and *M. harmandii*. The flowers of this plant have been used in traditional Thai medicine as a hearth tonic. Plants of this genus are known to be rich sources of coumarins (structures **1-19**) (Prachyawarakorn et al., 2006, Reutrakul et al., 2003, Mahidol et al., 2002, Kaweetripob et al., 2000, Prachyawarakorn et al., 2000, Tosa et al., 1997, Iinuma et al., 1996, Combie et al., 1987, Thebtaranonth et al., 1981, Crichton and Waterman 1978, Joshi et al., 1969) and xanthones (structures **20-26**) (Poobrasert et al., 1998, Tosa et al., 1997, Iinuma et al., 1996), with more than 30 compounds have been isolated from this genus.

Description of *M. siamensis* (Gutteferae)

The characteristics of this plant were summarized below:

Bark: pale grey-brown, smooth or slightly fissured, inner bark dark red with scant cream or pale yellow latex.

Leaf: 7.5-2.5 × 2.5-7 cm, obovate or oblong, with blunt or slightly notched tip & tapering base. Young leaves purple, mature leaves dark-green above, yellow-green below, completely smooth. Side veins numerous, slender but clearly visible on both surfaces. Stalks 0.5-1.5 cm.

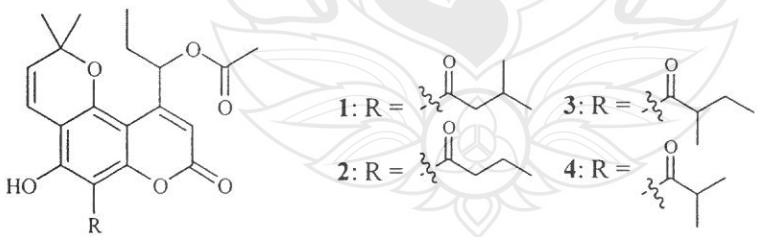
Flower: 1.2-2.5 cm, white or pale yellow, male & female flowers on different trees, clustered on old woody twigs behind leaves. Stalks slender, 2 cm. Calyx fused in bud, later splitting into 2 lobes, 0.2-0.7 cm. 4 oblong petals, 0.6-0.8 cm. 60-90 stamens, single short style with 2-lobed stigma.

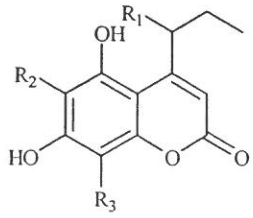
Fruit: 2.5-5 cm. yellow/orange, oval with short blunt tip, 2 valved, rind with sparse white latex, single large seed with thin yellow coating (aril).

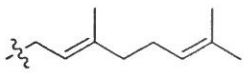
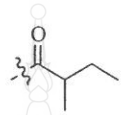
Thai medicinal uses: The flowers of this plant have been used as heart tonic.

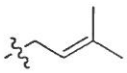
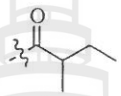
Fig. 1 *Mammea siamensis*

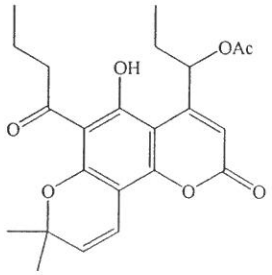
The structures of all isolated from this plant are summarized below:





5: R_1= OH, R_2 =  R_3 = 

6: R_1= OH, R_2 =  R_3 = 



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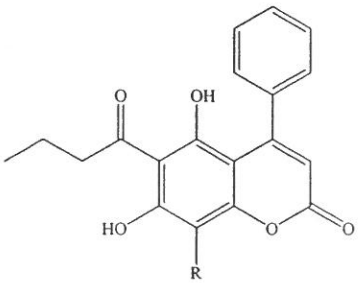

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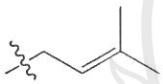
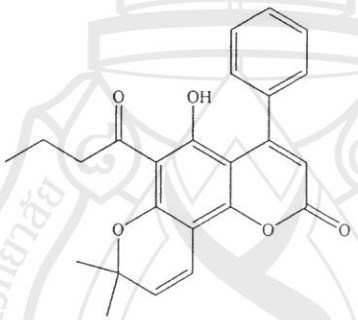
9: R= 



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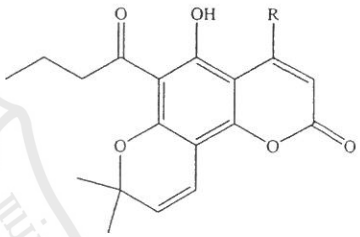

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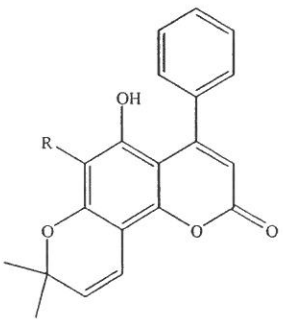
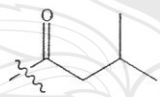
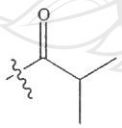
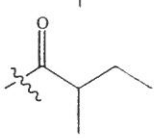
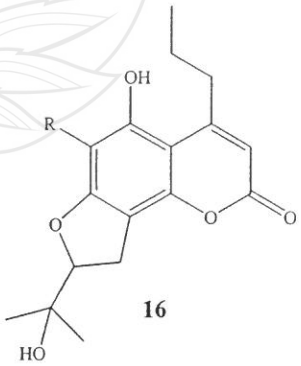


13: R = 

14: R = 

15: R = 



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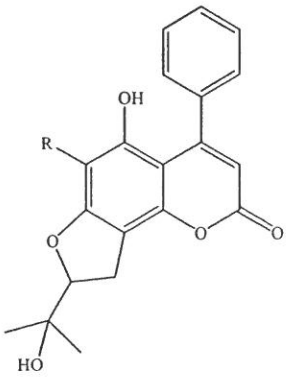
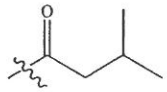
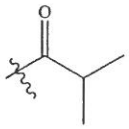
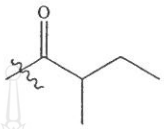
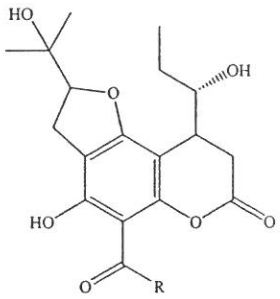


17: R = 

18: R = 

19: R = 



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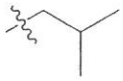
22: R = 

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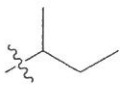
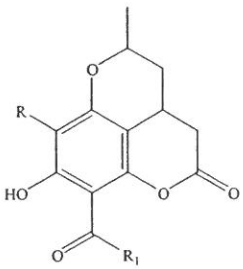
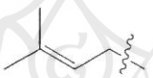
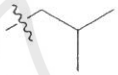
23: R = 



25: R = 

R_1 = 

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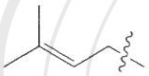
27: R = 

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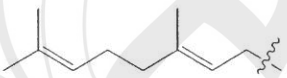
28: R = 

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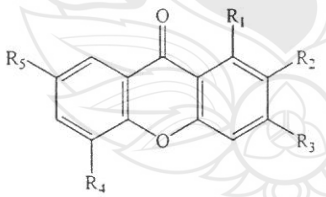


29: R_1=OMe; R_2= H; R_3=H; R_4=OH: R_5=H
30: R_1=OMe; R_2= H; R_3=OMe; R_4=OH: R_5=H
31: R_1=OMe; R_2= OMe; R_3=H; R_4=OH: R_5=H
32: R_1=OH; R_2= H; R_3=H; R_4=OH: R_5=OH
33: R_1=OMe; R_2= OH; R_3=H; R_4=OH: R_5=H
34: R_1=OH; R_2= H; R_3=OH; R_4=H: R_5=OH
35: R_1=OMe; R_2= H; R_3=OH; R_4=OH; R_5=H
36: R_1=OH; R_2= H; R_3=H; R_4=H: R_5=OH

CHAPTER 2

METHODOLOGY

2.1 General experimental procedures

Melting points were determined using the Fisher-John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. UV spectra were measured with a UV-160A spectrophotometers (Shimadzu). The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using 400 and/or 300 MHz Bruker FTNMR Ultra Shield™ spectrometers. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} was used for analytical purposes.

2.2 Plant material

The seeds of *M. siamensis* were collected from Mae Fah Luang University, Tasud, Muang, Chiang Rai Province, northern part of Thailand in August 2005. The identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a voucher specimen (No. SC09) was deposited at Prince of Songkla University Herbarium.

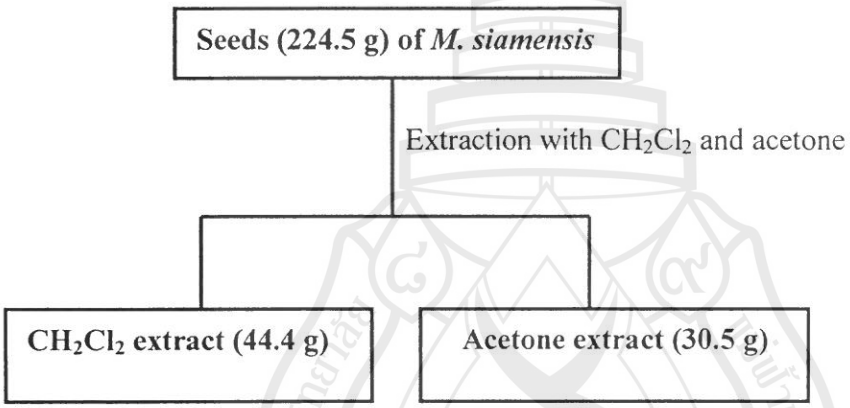
2.3 Cytotoxicity assay

The procedure for cytotoxic assay was performed by sulphorhodamine B (SRB) assay as described by Skehan et al. (Skehan at al., 1990). In this study, four cancer cell lines, MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) were used. Camptothecin, the reference substance,

exhibited activity toward MCF-7, HeLa, HT-29 and KB cell lines, with IC_{50} range of 0.2-2.0 μg mL^{-1} (Table 1).

2.4 Extraction

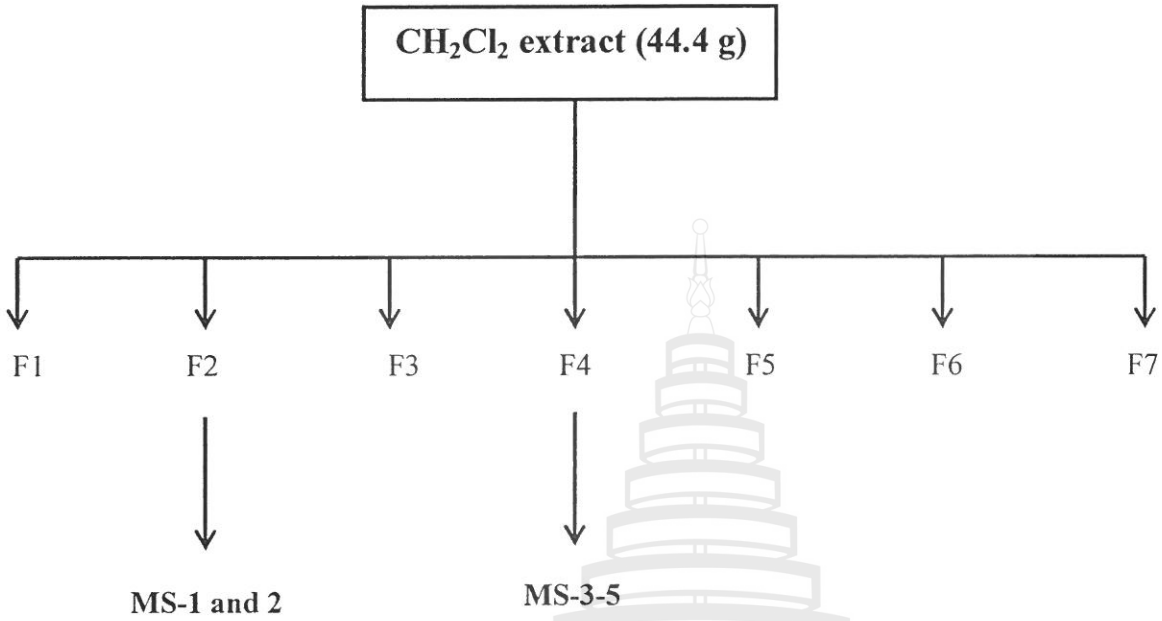
The seeds (224.5 g) of *M. siamensis* were extracted successively with CH_2Cl_2 (500 ml) and acetone at room temperature for 5 days. The filtered samples were combined and the solvents were evaporated under reduced pressure to provide the CH_2Cl_2 (44.4 g) and acetone extracts (30.5).



Scheme 1 Extraction of the seeds of *M. siamensis*

2.5 Isolation

The CH_2Cl_2 extract (44.4 g) was chromatographed by QCC and eluted with hexane–EtOAc mixtures to give seven fractions (F1-F7). Fraction F2 (1.92 g) was purified by RP-18 CC with acetone–H_2O (3:1) and followed by RP-18 preparative TLC with acetone: H_2O (3:1) to yield **MS-1** (3.1 mg) and **MS-2** (4.3 mg). Fraction F4 (3.35 g) was separated by CC with EtOAc–hexane (3:17) and followed by RP-18 preparative TLC with MeOH–H_2O (4:1) to afford five subfractions (F4a-F4e). Subfraction F4d (1.02 g) was purified by RP-18 CC with MeOH–H_2O (4:1) and followed by CC with EtOAc–hexane (1:3) to afford **MS-4** (16.8 mg), **MS-3** (32.6 mg) and **MS-5** (12.7 mg).

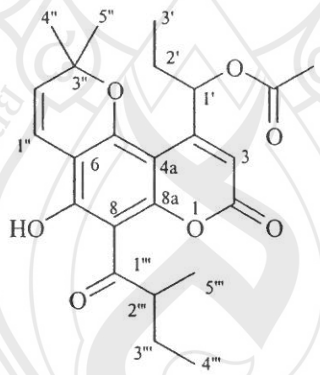

CH_2Cl_2 extract (44.4 g)
F1
F2
F3
F4
F5
F6
F7
MS-1 and 2
MS-3-5

CHAPTER 3

RESULTS AND DISCUSSION

The crude CH_2Cl_2 extract from the seed of *M. siamensis* was subjected to a succession of chromatographic procedures, including silica gel column chromatography and preparative TLC to afford two novel compounds, mammea E/BB cyclo D (**MS-1**), together with three known coumarins, mammea E/BC cyclo D (**MS-2**) (Mahido et al., 2002), suragin C (**MS-3**) (Mahandru et al., 1986), and therapin B (**MS-4**) (Lee et al., 2003).

3.1 MS-1 (Mammea E/BB cyclo D)



Mammea E/BB cyclo D (**MS-1**) was isolated as a yellowish viscous oil, with a molecular formula $C_{24}H_{28}O_7$, established by HREIMS analysis of its molecular ion $[M]^+$ at *m/z* 428.1813 (calcd for $C_{24}H_{28}O_7$ *m/z* 428.1835). The UV spectrum of **MS-1** showed absorption bands at 225, 280, 285, 300 and 373 nm suggesting the presence of conjugation in the molecule. The IR spectrum exhibited the characteristic of carbonyl (1738 and 1655 cm^{-1}) and hydroxyl (3454 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra revealed 24 carbons, including six methyls (δ 10.0, 10.6, 16.9, 21.1, 27.8 and 28.4), two methylenes (δ 28.6 and 29.6), five methines (δ 46.9, 73.0, 106.4, 115.8 and

126.8) and eleven non-hydrogenated carbons (δ 80.2, 100.9, 103.7, 106.5, 155.7, 156.7, 157.5, 159.3, 163.5, 170.3 and 210.8). The ^{1}H NMR spectral data (**Table** 1) showed a chelated hydroxyl proton at δ 14.44 assignable to 7-OH on the basis of HMBC correlations (**Figure** 2). The ^{1}H NMR spectrum also displayed a singlet signal at δ 6.30, which is a typical chemical shift for H-3 of 4-alkylcoumarin skeleton (Mahidol et al., 2002, Cruz et al., 2001). In addition, the ^{1}H NMR spectrum also showed the signals of chromene ring, 2-methyl-1-oxobutyl and 1-acetoxypropyl moieties. The ^{1}H NMR signals of chromene ring were appeared at δ 6.74 (1H, d, J 10.0 Hz, H-1″), 5.60 (1H, d, J 10.0 Hz, H-2″), 1.58 (3H, s, H-4″) and 1.56 (3H, s, H-5″), while the 2-methyl-1-oxobutyl group showed signals at δ 4.02 (1H, sextet, J 6.3 Hz, H-2‴), 1.80 (1H, m, H-3‴a), 1.45 (1H, m, H-3‴b), 1.26 (3H, d, J 6.3 Hz, H-5‴) and 1.06 (3H, t, J 7.2 Hz, H-4‴). Finally, the 1-acetonylpropyl moiety showed the ^{1}H NMR signals at δ 6.60 (1H, dd, J 6.8, 2.8 Hz, H-1′), 2.17 (3H, s, H-1′-COCH_3), 1.97 (1H, m, H-2′a), 1.78 (1H, m, H-2′b), and 1.07 (3H, t, J 7.2 Hz, H-3′). The locations of the three moieties were established based on the observed key HMBC correlations (Figure 2). The 1-acetoxypropyl unit was placed at C-4 due to the oxymethine proton H-1′ (δ 6.60) showed ^2J and ^3J correlation with C-4a (δ 100.9), C-4 (δ 157.5) and C-3 (δ 106.4) in the HMBC spectrum. In addition, the olefinic proton H-3 (δ 6.30) also showed ^2J and ^3J correlations with C-1′ (δ 73.0), C-2 (δ 159.3) and C-4a (δ 100.9). The chromene ring was located at C-5/C-6 because the olefinic proton H-4″ (δ 6.74) displayed HMBC correlations to C-5 (δ 155.7), C-6 (δ 106.5) and C-7 (δ 163.5). Finally, the hydroxyl group was located at C-4 because the chelated hydroxyl proton showed HMBC correlations to C-6 (δ 106.5), C-7 (δ 163.5) and C-8 (δ 103.7) and the 2-methyl-1-oxobutyl moiety had to be placed at C-8 by process of elimination. Therefore, the structure of mammea E/BB cyclo D was characterized as **MS-1**.

Table 1 ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectral data of **MS-1** ($CDCl_3$)

Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
2	159.3	–	2″	126.8	5.60 *d* (10.0)
3	106.4	6.30 *s*	3″	80.2	–
4	157.5	–	4″	28.4	1.58 *s*
4a	100.9	–	5″	27.8	1.56 *s*
5	155.7	–	1‴	210.8	–
6	106.5	–	2‴	40.3	4.02 *sextet* (6.3)
7	163.5	–	3‴	19.2	1.26 *d* (6.3)
8	103.7	–	4‴	29.6	1.45 *m*; 1.80 *m*
8a	156.7	–	5‴	19.2	1.06 *t* (7.2)
1′	73.0	6.60 *dd* (6.8, 2.8)	*CH*$_3$CO	21.0	2.17 *s*
2′	28.6	1.78 *m*; 1.97 *m*	CH$_3$*C*O	170.3	–
3′	10.0	1.07 *t* (7.2)	7-OH	–	14.44 *s*
1″	115.8	6.74 *d* (10.0)			

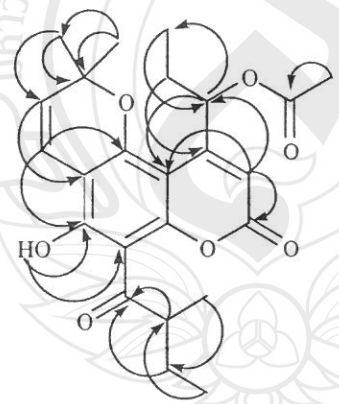
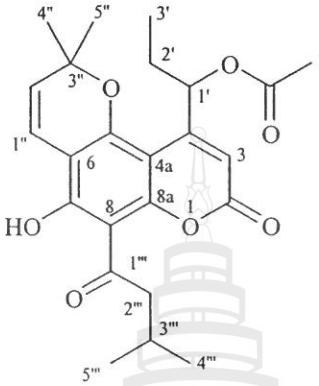


Figure 2 HMBC Correlation of **MS-1**

3.2 MS-2 (Mammea E/BA cyclo D)



MS-2 was isolated as yellowish viscous oil. The ^{1}H and ^{13}C NMR spectra of **MS-2** (**Table** 2) were similar to those of **MS-1**, except that **MS-2** showed a 1-acetonyl-3-methylbutyryl group in stead of 1-acetonylpropyl moiety of **MS-1**. Therefore, **MS-2** was identified as mammea E/BA cyclo D. This compound has been isolated from the flowers of this plant by Mahidol et al. in 2002.

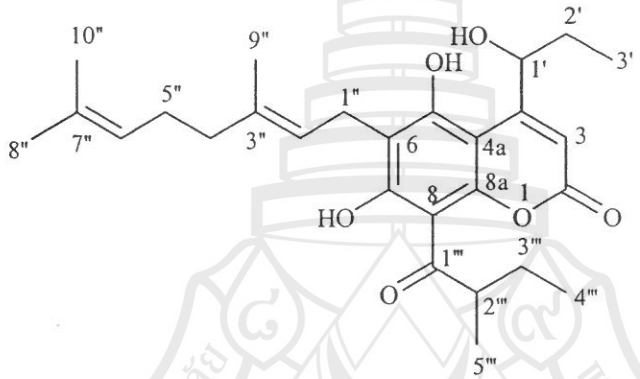
Table 2 ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectral data of **MS-2** ($CDCl_3$)

Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
2	159.2	–	2′	28.6	
3	106.8	6.30 *s*	3′	10.0	1.09 *t* (7.2)
4	157.3	–	1″	115.8	6.74 *d* (10.0)
4a	100.8	–	2″	126.8	5.60 *d* (10.0)
5	155.8	–	3″	80.2	–
6	106.4	–	4″	28.4	1.58 *s*
7	163.3	–	5″	27.8	1.56 *s*
8	104.6	–	1‴	206.2	–
8a	157.0	–	2‴	53.6	3.18, *m*
1′	73.0	6.60 *dd* (6.8, 2.8)	3‴	25.5	2.28, *m*

Table 2 (Continued)

Position	δ_C	δ_H (*J* in Hz)		Position	δ_C	δ_H (*J* in Hz)
4‴	22.6	1.03 *d* (6.7)		CH_3CO	170.3	–
5‴	22.6	1.02 *d* (6.7)		7-OH		14.52 *s*
CH_3CO	21.0	2.20 *s*				

3.3 MS-3 (Suragin C)



MS-3 was isolated as yellowish viscous oil. It gave a molecular ion by HREIMS at *m/z* 456.2501 $[M]^+$, corresponding to the molecular formula $C_{27}H_{36}O_6$ (calcd for $C_{27}H_{36}O_6$ *m/z* 456.2512). The EIMS spectrum showed fragment ions at *m/z* 455 $[M-H]^+$, 437 $[455-H_2O]^+$, 314 $[437-C_9H_{15}]^+$, 256 $[314-C_4H_9]^+$, and 228 $[256-CO]^+$. The ^{13}C NMR and DEPT spectra revealed 27 carbons (**Table** 3), including six methyls, five methylenes, five methines and eleven quaternary carbons. The ^1H NMR spectral data (**Table** 3) showed signals corresponding to 4-alkylcoumarin skeleton. The ^1H NMR spectra revealed two chelated hydroxyl protons at δ 13.90 and 11.20 assignable to 7-OH and 5-OH, respectively. The ^1H NMR spectrum also displayed a singlet signal at δ 6.05, which was identified as a characteristic of H-3 of 4-alkylcoumarin skeleton. With combination of the ^1H-^1H COSY spectrum, the presence of three partial structural units characterized as 2-methyl-1-oxobutyl, hydroxypropyl and geranyl groups were revealed. The 2-methyl-

1-oxobutyl moiety was characterized by the resonances of protons at δ 3.66 (1H, *sextet*, J = 6.9 Hz, H-2‴), 1.85 (1H, *m*, H-4‴a), 1.43 (1H, *m*, H-4‴b), 1.19 (3H, *d*, J = 6.9 Hz, H-3‴) and 0.94 (3H, *t*, J = 7.2 Hz, H-5‴), while those of the hydroxypropyl group were found at δ 4.69 (1H, *dd*, J = 6.3, 6.3 Hz, H-1′), 1.95 (1H, *m*, H-2′a), 1.84 (1H, *m*, H-2′b) and 1.01 (3H, *t*, J = 7.5 Hz, H-3′). ^{1}H NMR signals of the geranyl side chain were observed at δ 5.20 (1H, *m*, H-2″), 5.05 (1H, *m*, H-6″), 3.40 (2H, *dd*, J = 1.2, 6.6 Hz, H-1″), 2.00 (2H, *m*, H-4″), 2.00 (2H, *m*, H-5″), 1.79 (3H, *s*, H-9″), 1.63 (3H, *s*, H-8″) and 1.56 (3H, *s*, H-10″). These assignments were confirmed by HMBC correlations (**Figure** 3). The locations of the three side chains were established based on the observed key HMBC correlations. The hydroxypropyl unit was placed at C-4 from HMBC correlations of the oxymethine proton at δ 4.69 (H-1′) with the resonances at C-4a (δ 100.8), C-4 (δ 156.1) and C-3 (δ 108.6) and of the singlet olefinic proton at δ 6.05 (H-3) with C-1′ (δ 77.6), C-2 (δ 160.5) and C-4a (δ 100.8). The geranyl unit was located at C-6 because the methylene protons at δ 3.40 (2H-1″) displayed HMBC correlations to the signals of C-7 (δ 166.4), C-6 (δ 114.2) and C-5 (δ 156.8). Finally, the 2-methyl-1-oxobutyl moiety was placed at C-8 by process of elimination. Therefore, the structure of suragin C was characterized as **MS-3**.

Table 3 ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectral data of **MS-3** ($CDCl_3$)

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
2	160.5		8	104.0	–
3	108.6	6.05 *s*	8a	157.8	–
4	156.1	–	1′	77.6	4.69 *dd* (6.3, 6.3)
4a	100.8	–	2′	26.9	1.84 *m*; 1.95 *m*
5	156.8	–	3′	11.6	1.01 *t* (7.5)
6	114.2	–	1″	22.6	3.40 *dd* (6.6, 1.2)
7	166.4	–	2″	121.1	5.20 *m*

Table 3 (Continued)

Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
3″	136.8	–	1‴	210.1	–
4″	39.7	2.00 *m*	2‴	47.0	3.66 *sextet* (6.9)
5″	27.6	2.00 *m*	3‴	16.6	1.19 *d* (6.9)
6″	124.4	5.05 *m*	4‴	27.8	1.43 *m*;1.85 *m*
7″	131.3	–	5‴	10.5	0.94 *t* (7.2)
8″	17.6	1.63 *s*	5-OH		11.20 *br s*
9″	26.6	1.56 *s*	7-OH		13.90 *s*
10″	16.21	1.79 *s*			

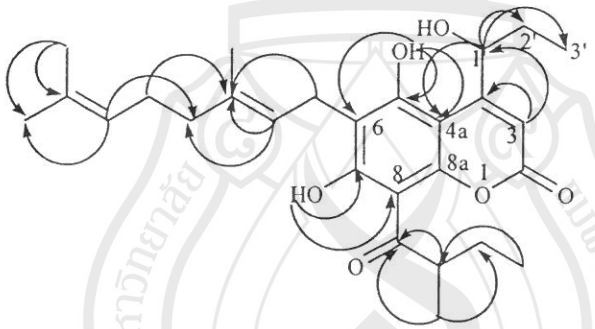
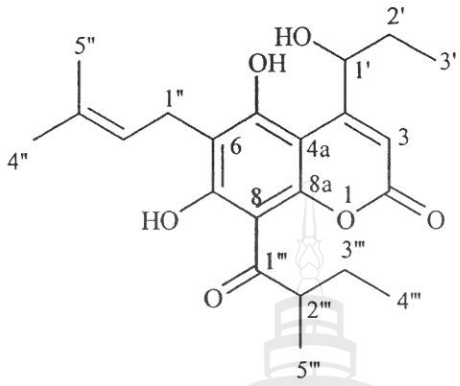


Figure 3 HMBC Correlation of **MS-3**

3.4 MS-4 (Therapin B)



MS-4 was isolated as a yellowish viscous oil, with a molecular formula of $C_{22}H_{28}O_6$ as established by HREIMS analysis of its molecular ion4 $[M]^+$ at *m/z* 388.1894 (calcd *m/z* 388.1886). The ^1H and ^{13}C NMR spectra of **MS-4** (**Table** 4) were similar to those of **MS-3**, except that **MS-4** showed a prenyl group in stead of geranyl group of **MS-3**. In addition, the structure of **MS-3** was also confirmed by HMBC correlations (**Figure** 4). Therefore, **MS-4** was identified as theraphin B. This compound has been isolated from *Kayea assamica* by Lee et al. in 2003.

Table 4 ^1H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectral data of **MS-4** ($CDCl_3$)

Position	δ_C	δ_H (*J* in Hz)	**Position**	δ_C	δ_H (*J* in Hz)
2	160.1		1′	77.8	4.67 *t* (7.2)
3	108.8	6.05 *s*	2′	26.9	1.84 *m*; 1.95 *m*
4	156.1	–	3′	11.6	1.01 *t* (7.5)
4a	100.8	–	1″	22.0	3.38 *br d* (6.0)
5	156.4	–	2″	121.3	5.21 *m*
6	114.2	–	4″	25.8	1.69 *s*
7	166.4	–	5″	17.9	1.80 *s*
8	103.9	–	1‴	210.1	–
8a	157.7	–	2‴	47.0	3.70 *sextet* (6.6)

Table 4 (Continued)

Position	δ_C	δ_H (*J* in Hz)		Position	δ_C	δ_H (*J* in Hz)
3‴	16.6	1.19 *d* (6.6)		5-OH		11.19 *s*
4‴	27.8	1.43 *m*; 1.85 *m*		7-OH		14.19 *s*
5‴	10.5	0.93 *t* (7.5)				

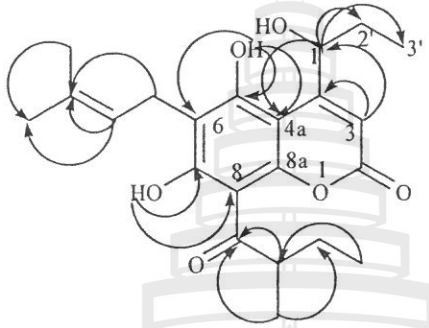
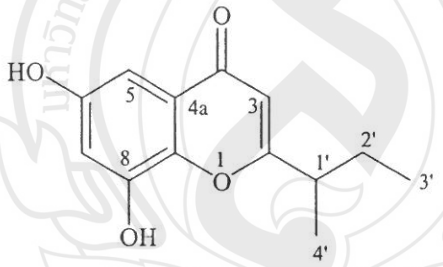


Figure 4 HMBC Correlation of **MS-4**

3.5 MS-5 (Siamensone A)



Siamensone A (**MS-5**), yellowish solid, is a 6,8-dihydroxy-2-*sec*-butyl-4*H*-chromen-4-one. Its molecular formula of $C_{13}H_{14}O_4$ with a molecular ion $[M]^+$ at *m/z* 234.0877 (calc. $C_{13}H_{14}O_4$ *m/z* 234.0892) was established by HREIMS analysis. This compound exhibited UV absorption maxima at 225, 248, 300 and 337 nm, suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of OH stretching at 3399 cm^{-1} and C=O stretching at 1714 cm^{-1}. The ^{13}C NMR spectrum also showed the resonance of a carbonyl carbon at δ 182.7 (C–4). The ^1H NMR spectrum

(**Table** 5) displayed the characteristic signals of *meta*-coupled aromatic protons at δ 6.32 (*d*, *J* = 2.1 Hz, H–5) and 6.24 (*d*, *J* = 2.1 Hz, H–7) and a singlet signal of an olefinic proton at δ 6.00 (*s*, H–3). With combination of the COSY spectrum, a *sec*-butyl moiety was evident from ^{1}H NMR signals at δ 2.58 (*sextet*, *J* = 6.9 Hz, H–1′), 1.70-1.80 (*m*, H–2′a), 1.55-1.65 (*m*, H–2′b), 1.27 (d, *J* = 6.9 Hz, Me–4′) and 0.93 (*t*, *J* = 7.5 Hz, Me–3′). The *sec*-butyl moiety was located at C–2 position based on HMBC correlations (**Table** 5). The singlet methine proton signal at δ 6.00 (H–3) correlated to C–1′ (40.4) and a sextet methine proton at δ 2.58 (H–1′) correlated to C–2 (174.2) and C–3 (106.7). The other HMBC correlations were summarized in Table 1. Therefore, **MS-5** was deduced to be siamensone A.

Table 5 ^{1}H- NMR (300 MHz), ^{13}C-NMR (75 MHz), COSY and HMBC spectral data of **MS-5** in $CDCl_3$

C/H	δ_C	δ_H (*J* in Hz)	^{1}H–^{1}H COSY	HMBC Correlations ^{1}H→^{13}C
2	174.2			
3	106.7	6.00 (s)		C–2, C–4, C–4a, C–1′
4	182.7			
4a	105.0			
5	94.2	6.32 (d, *J* = 2.1)	H–7	C–4, C–4a, C–6, C–7, C–8a
6	163.2			
7	99.0	6.24 (d, *J* = 2.1)	H–5	C–5, C–6, C–8, C–8a
8	161.8			
8a	158.3			
1′	40.4	2.58 (sextet, *J* = 6.9)	H–2′, H–4′	C–2, C–3, C–2′, C–3′, C–4′
2′	27.5	1.70 –1.80 (m); 1.55 –1.65 (m)	H–1′, H–3′	C–2, C–1′, C–3′, C–4′
3′	11.5	0.93 (t, *J* = 7.5)	H–2′	C–1′, C–2′
4′	17.7	1.27 (d, *J* = 6.9)	H–1′	C–1′, C–2′

3.5 Cytotoxic activity

The reported compounds were tested for their cytotoxicity against MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) cell lines. The results are summarized in **Table** 6. Suragin C (**MS-3**) showed cytotoxic activities against all four cancer cell lines better than therapin B (**MS-4**) (**Table** 5), while the remaining compounds were found to be inactive. It is interesting to note that the structural difference between suragin C (**MS-3**) and therapin B (**MS-4**) is only at C-6 (**MS-3** possesses a geranyl group while **MS-4** contains a prenyl group). The presence of a geranyl moiety seems to be important for enhancing the cytotoxic activity. The anticancer drug used as a standard in our cytotoxic assay is camptothecin, which has an IC_{50} in the range of 0.2-2.0 μg/ml.

Table 6 Cytotoxic Activity of **MS-1-5**

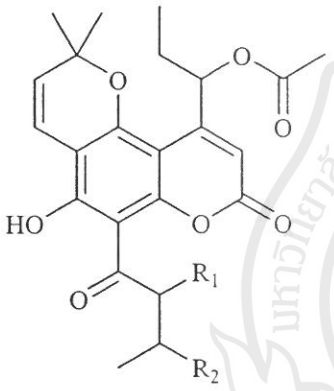
Compound	IC_{50} (μg/mL)			
	MCF-7[a]	Hela[b]	HT-29[c]	KB[d]
MS-1	Inactive[e]	Inactive[e]	Inactive[e]	Inactive[e]
MS-2	Inactive[e]	Inactive[e]	Inactive[e]	Inactive[e]
MS-3	1.33	2.56	0.78	1.33
MS-4	4.64	3.52	4.06	4.06
MS-5	Inactive[e]	Inactive[e]	Inactive[e]	Inactive[e]
Camptothecin	0.2-2.0	0.2-2.0	0.2-2.0	0.2-2.0

[a] MCF-7 (breast adenocarcinoma), [b] HeLa (human cervical cancer), [c] HT-29 (colon cancer) and [d] KB (human oral cancer) and [e] inactive at >20 μg/mL.

CHAPTER 4

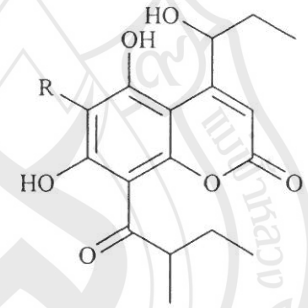
CONCLUSION

Two new phenolic compounds, mammea E/BB cyclo D (**MS-1**) and siamensone A (**MS-5**), together with three known coumarins, mammea E/BA cyclo D (**MS-2**), suragin C (**MS-3**) and therapin B (**MS-4**) were isolated from the seeds of *M. siamensis*. Their structures were characterized using 1D and 2D NMR spectral data. Suragin C (**MS-3**) and therapin B (**MS-4**) showed cytotoxic activity against breast adenocarcinoma (MCF-7), human cervical cancer (HeLa), colon cancer (HT-29) and human oral cancer (KB).

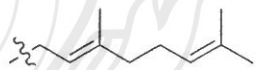


MS-1: $R_1 = CH_3$; $R_2 = H$

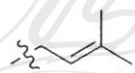
MS-2: $R_1 = H$; $R_2 = CH_3$

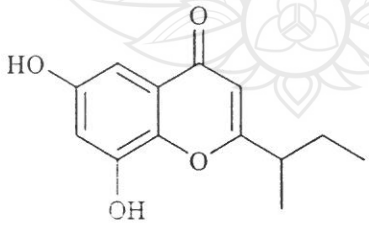


MS-3: R =



MS-4: R =





MS-5

It is worth noting that the genus *Mammea* of the family Guttiferae has been known to be rich in coumarins (Prachyawarakorn et al., 2006, Reutrakul et al., 2003, Mahidol et al., 2002, Kaweetripob et al., 2000, Prachyawarakorn et al., 2000, Tosa et al., 1997, Iinuma et al., 1996, Combie et al., 1987, Thebtaranonth et al., 1981, Crichton and Waterman 1978, Joshi et al., 1969) and xanthones (Poobrasert et al., 1998, Tosa et al., 1997, Iinuma et al., 1996), with more than 30 compounds have been isolated from this genus. In this study, we have been observed an additional new coumarin from the seeds of *M. siamensis*.

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APPENDIX

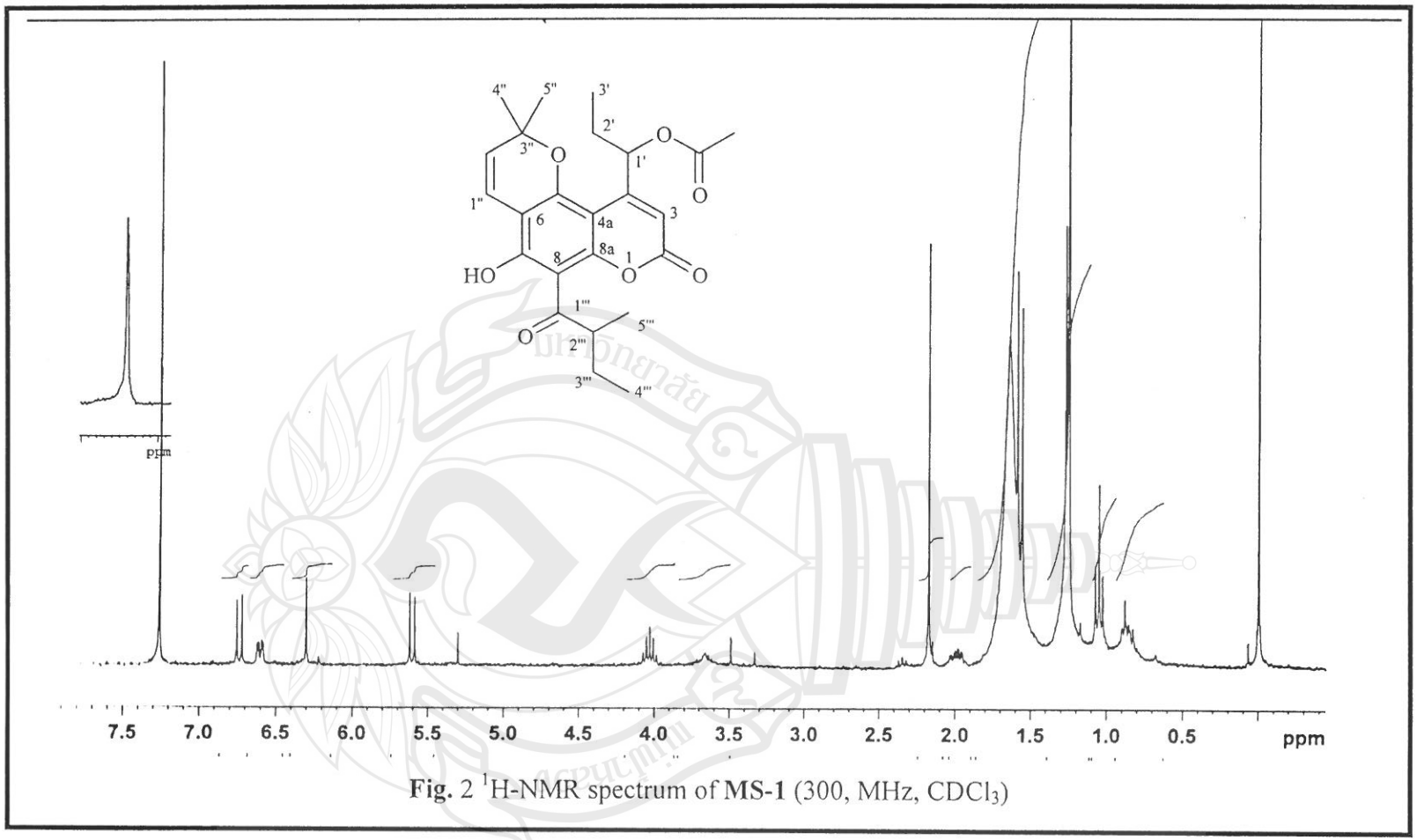

Fig. 2 ^{1}H-NMR spectrum of **MS-1** (300, MHz, $CDCl_3$)

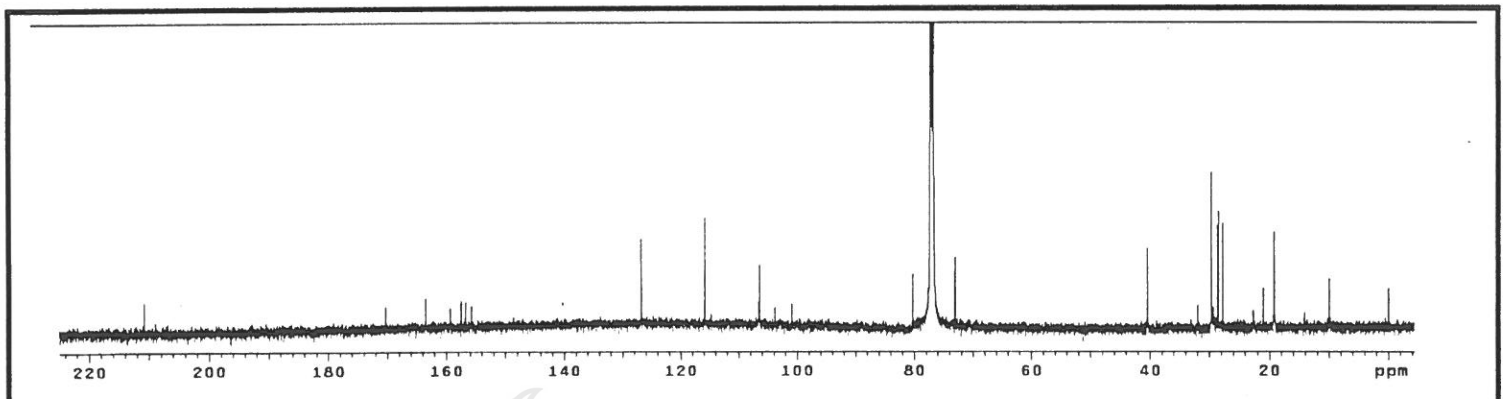

Fig. 3 ^{13}C-NMR spectrum of **MS-1** (125, MHz, $CDCl_3$)

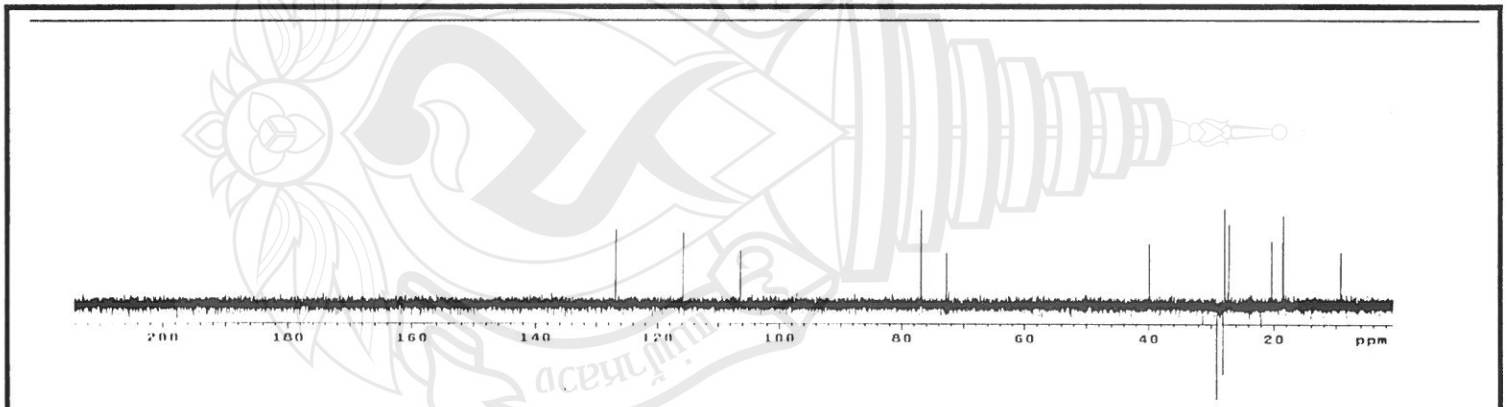

Fig. 4 DEPT 135° spectrum of **MS-1** (125, MHz, $CDCl_3$)

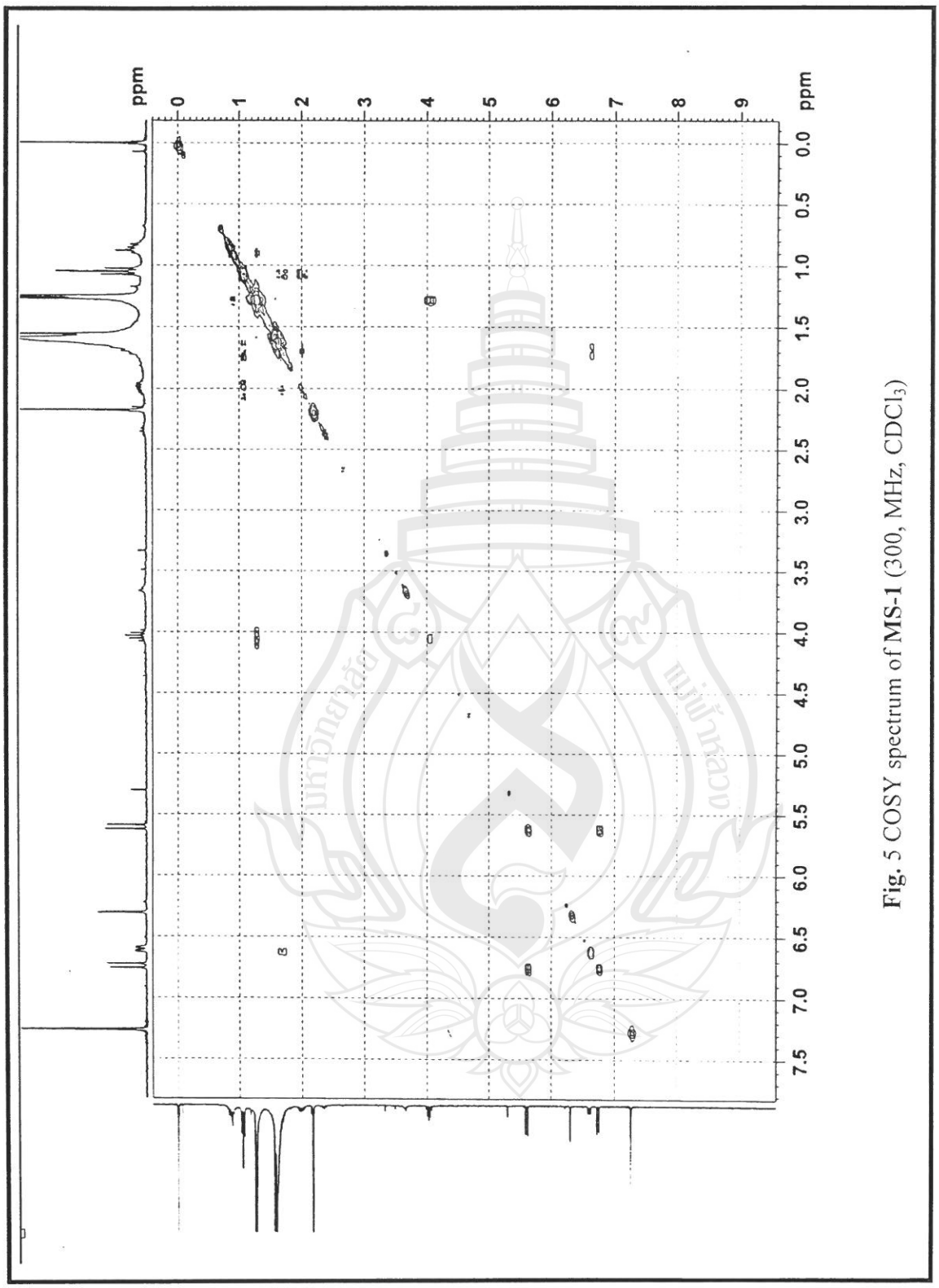

Fig. 5 COSY spectrum of **MS-1** (300, MHz, $CDCl_3$)

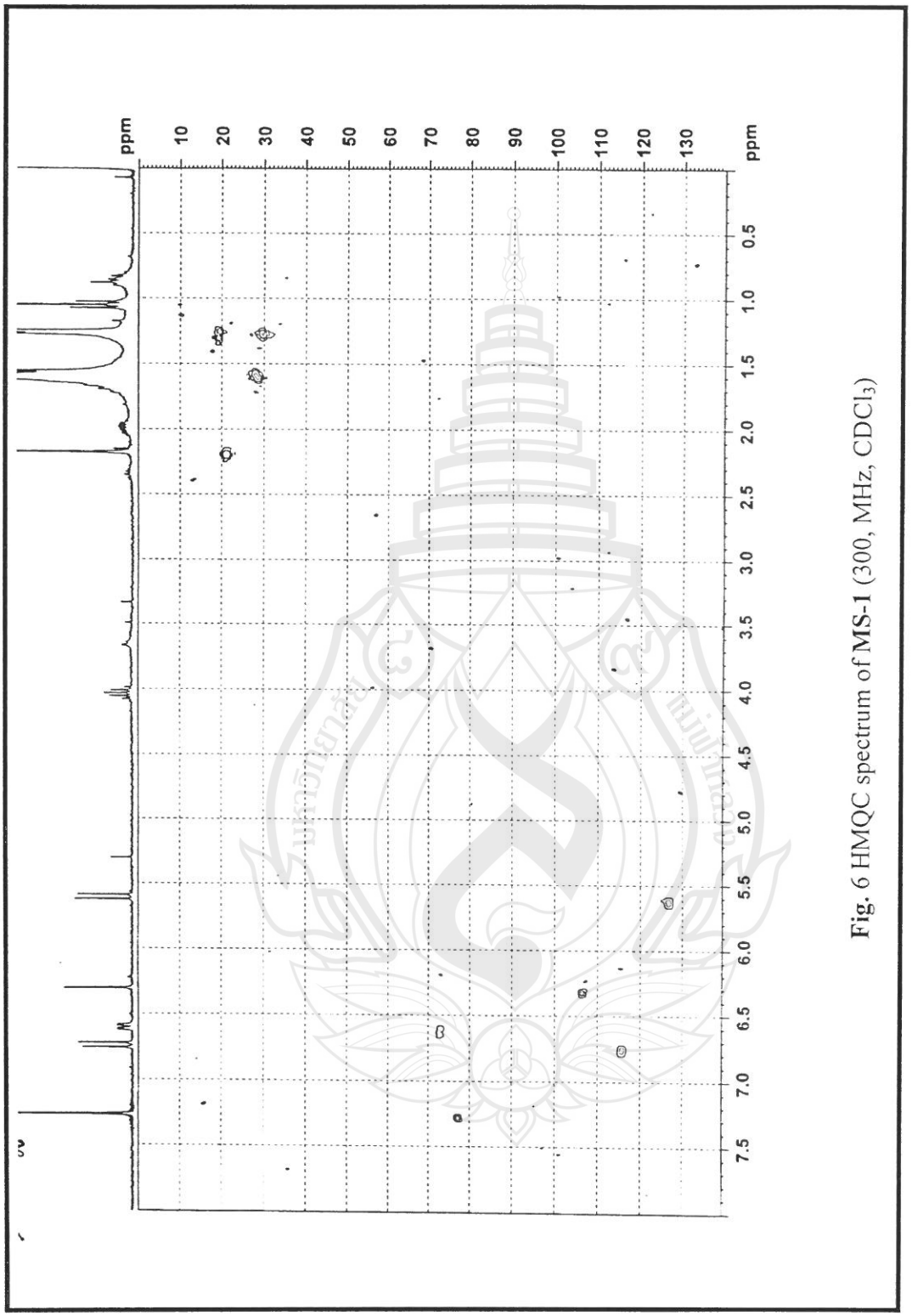

Fig. 6 HMQC spectrum of **MS-1** (300, MHz, $CDCl_3$)

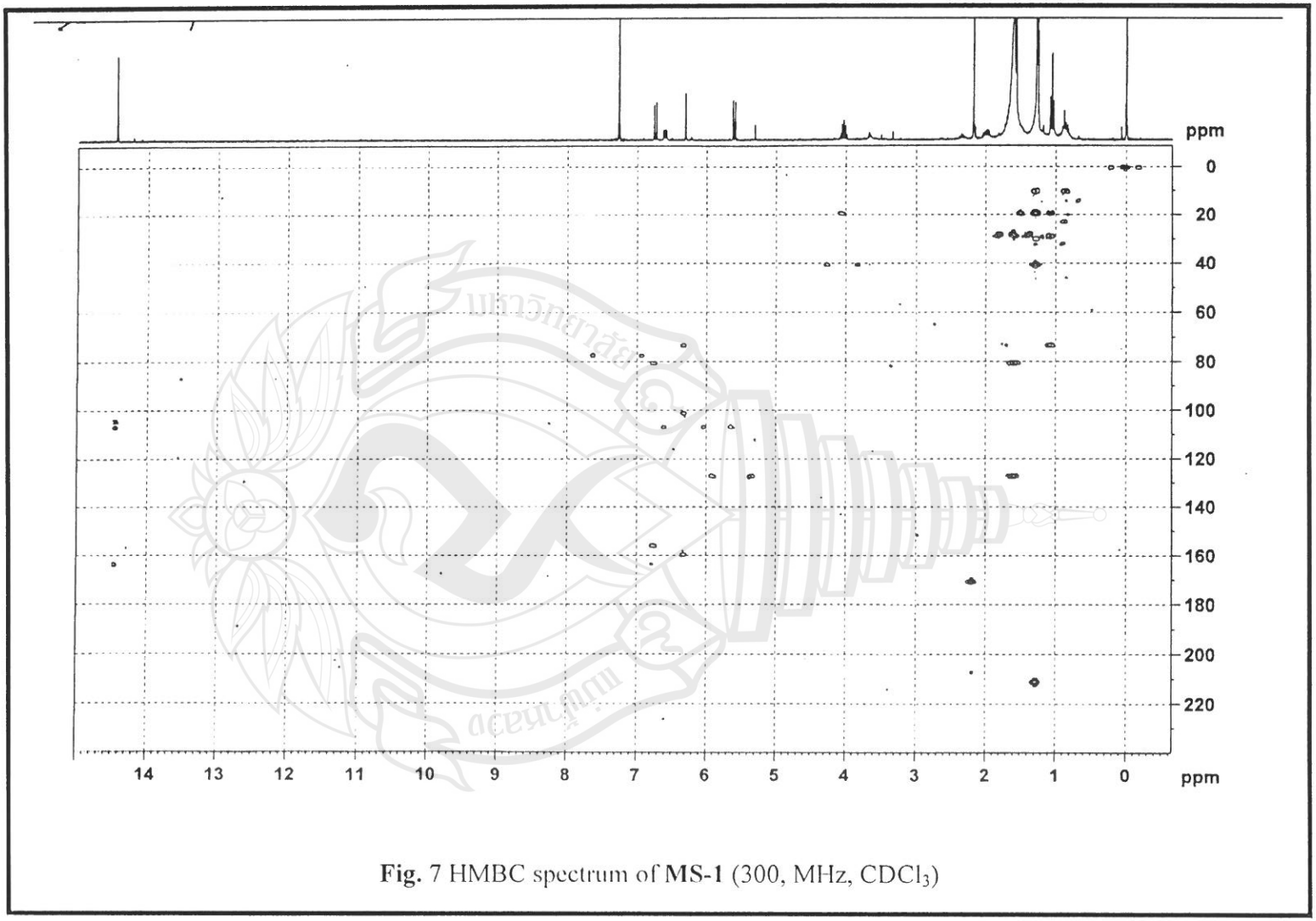

Fig. 7 HMBC spectrum of **MS-1** (300, MHz, $CDCl_3$)

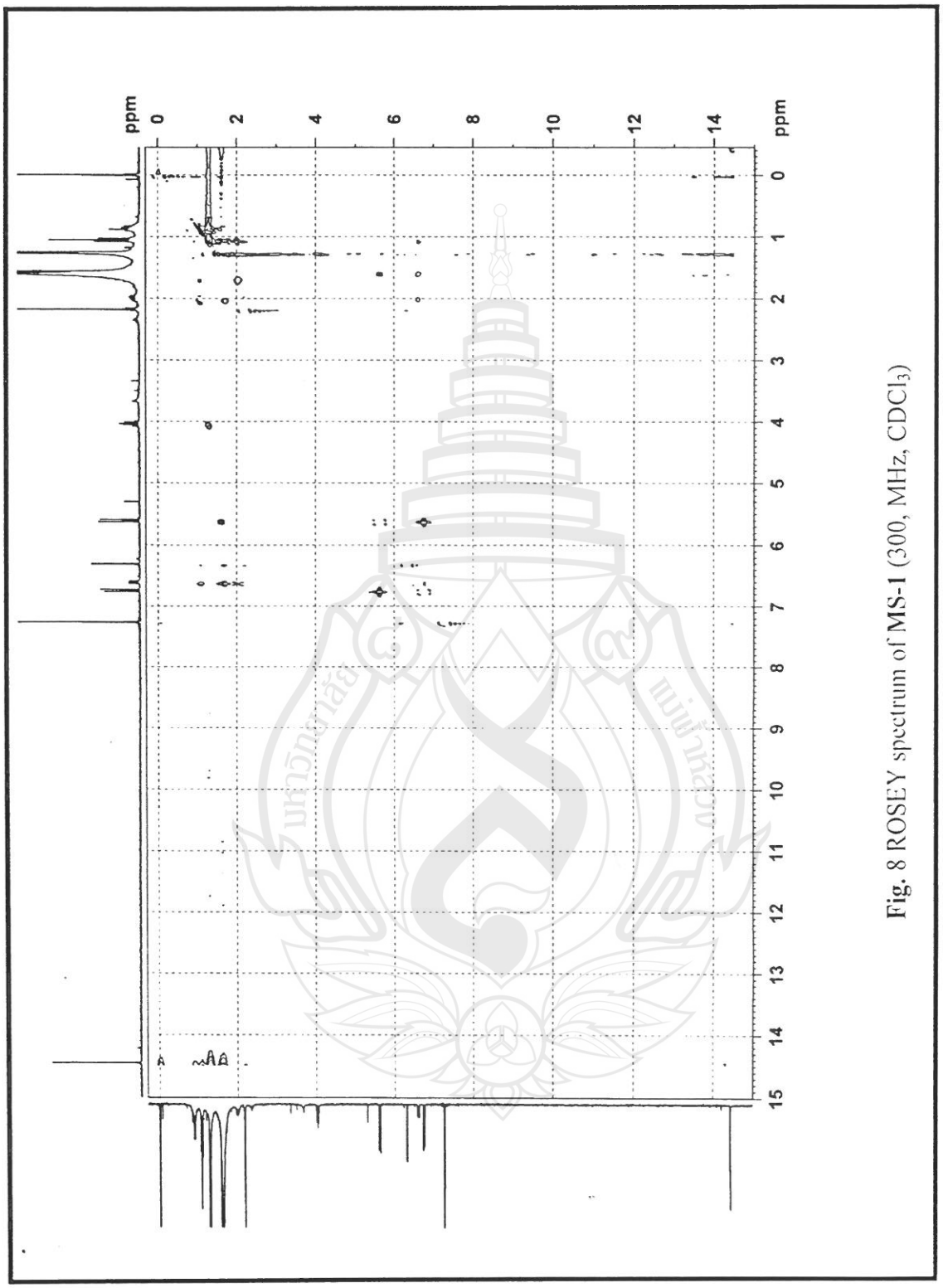

Fig. 8 ROSEY spectrum of **MS-1** (300, MHz, $CDCl_3$)

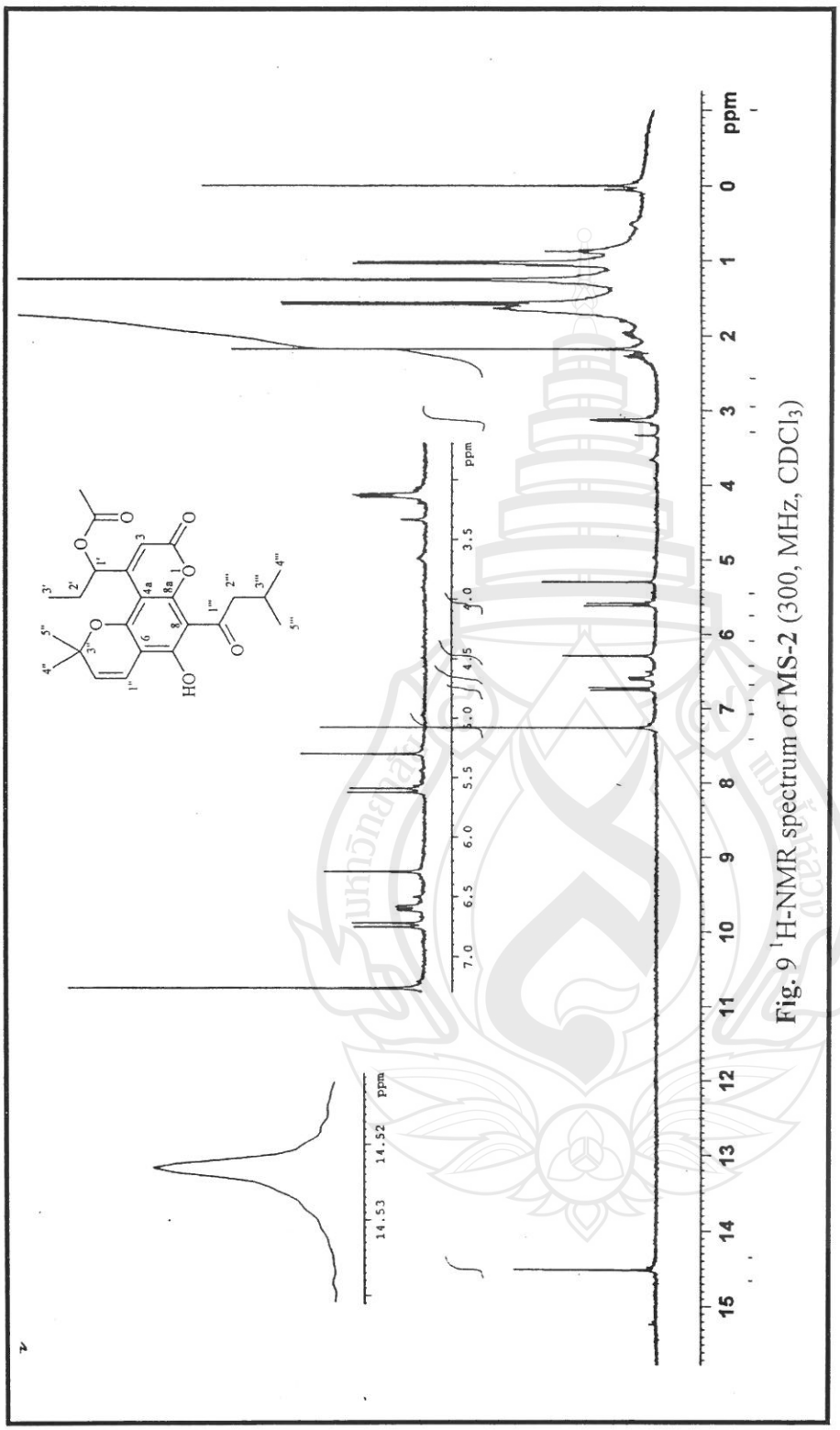

Fig. 9 ^{1}H-NMR spectrum of **MS-2** (300, MHz, $CDCl_3$)

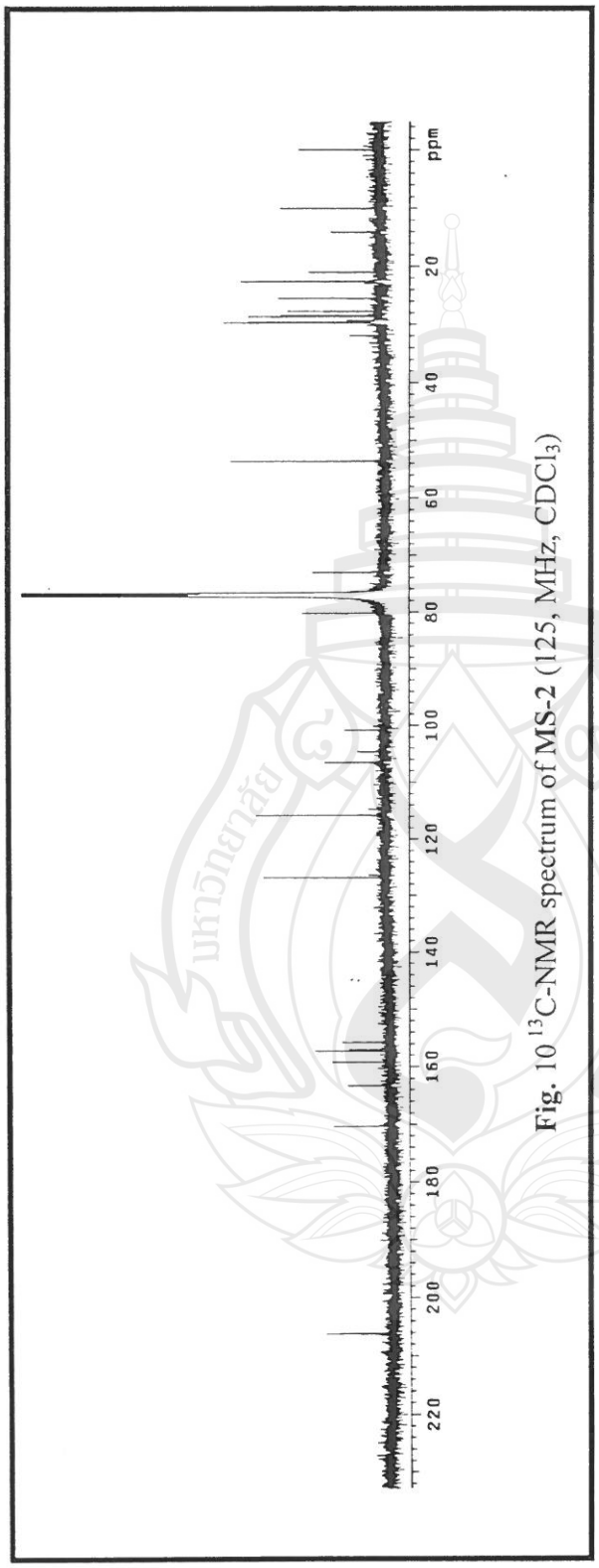

Fig. 10 ^{13}C-NMR spectrum of **MS-2** (125, MHz, $CDCl_3$)

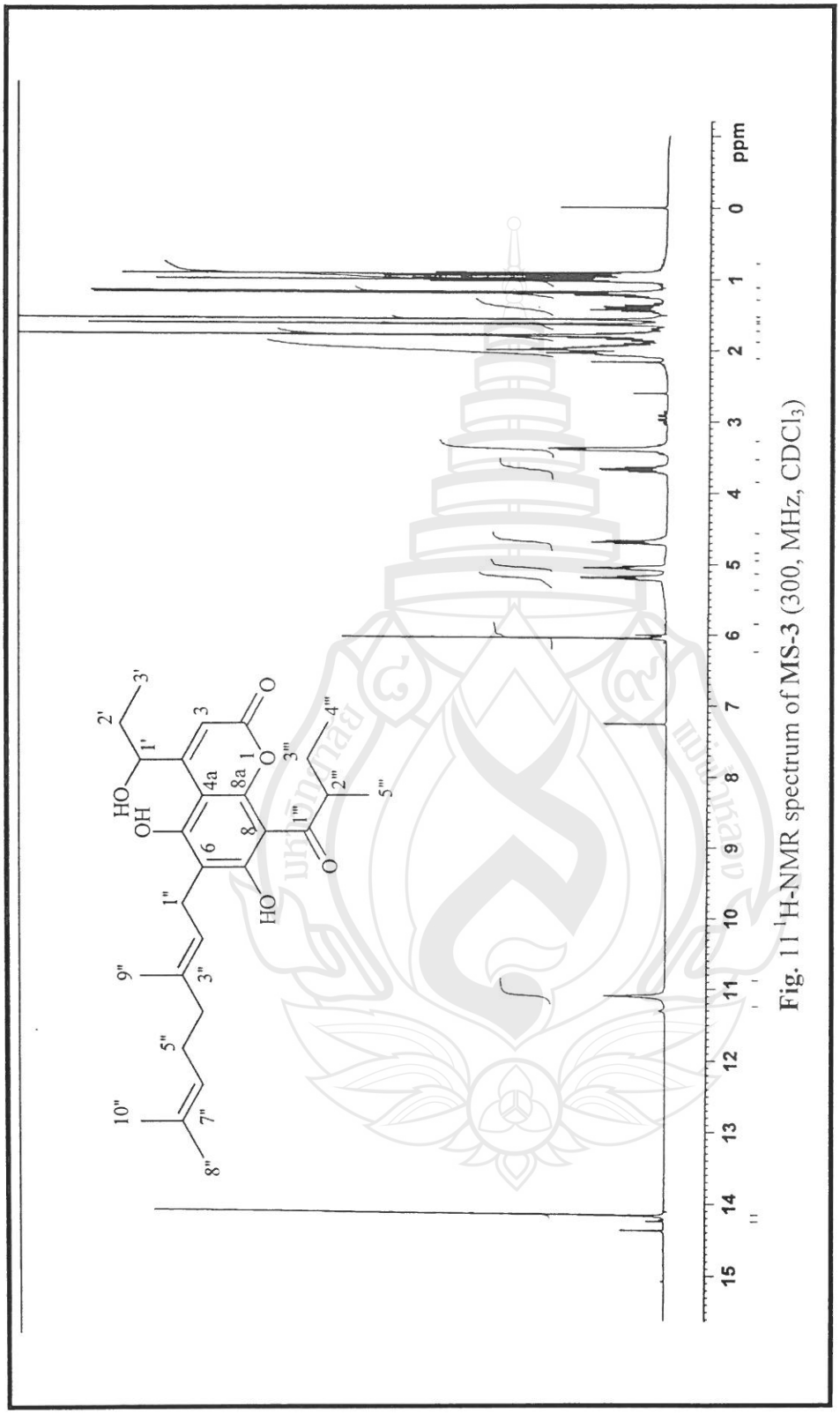

Fig. 11 ^{1}H-NMR spectrum of **MS-3** (300, MHz, $CDCl_3$)

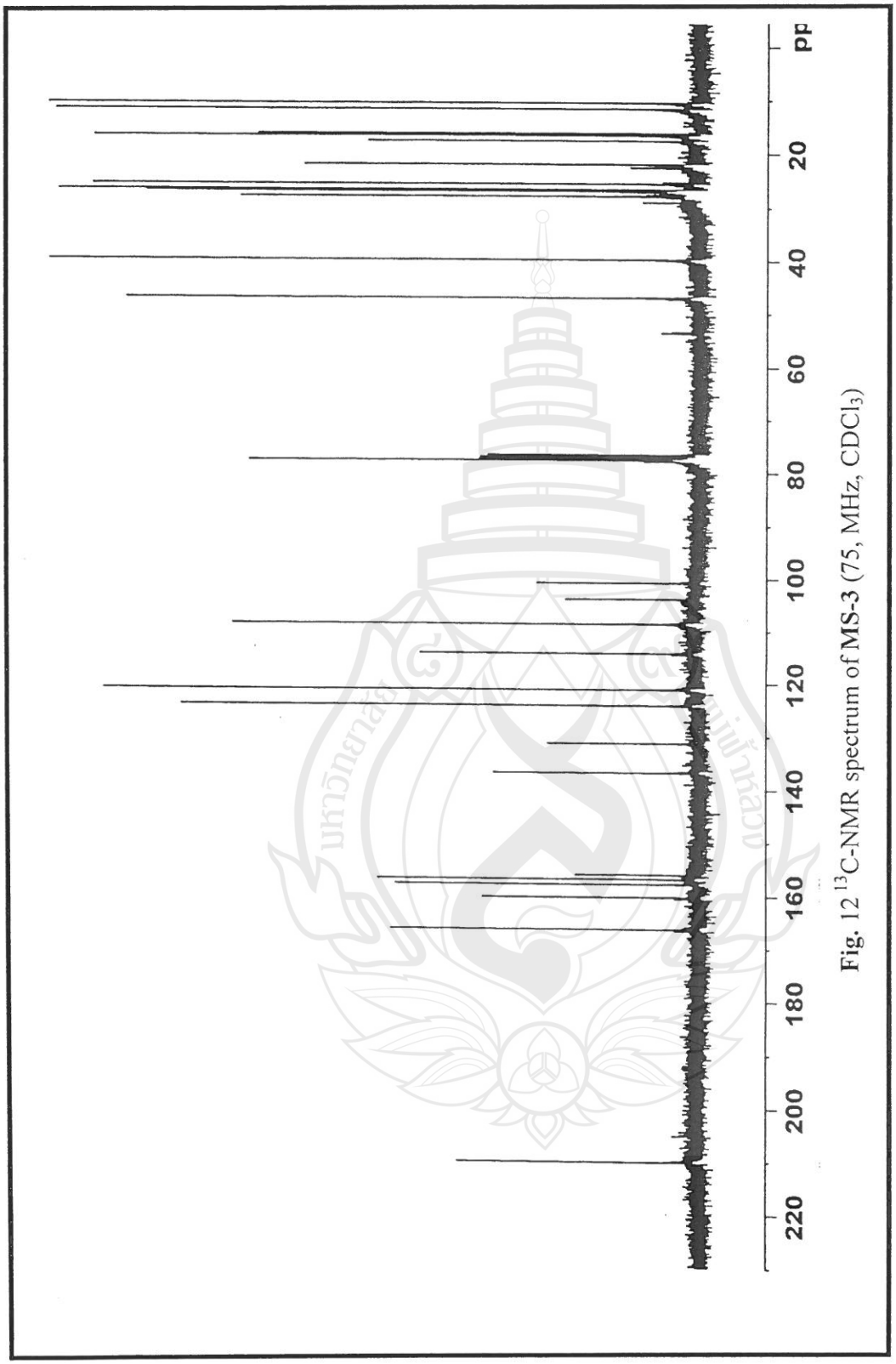

Fig. 12 ^{13}C-NMR spectrum of **MS-3** (75, MHz, $CDCl_3$)

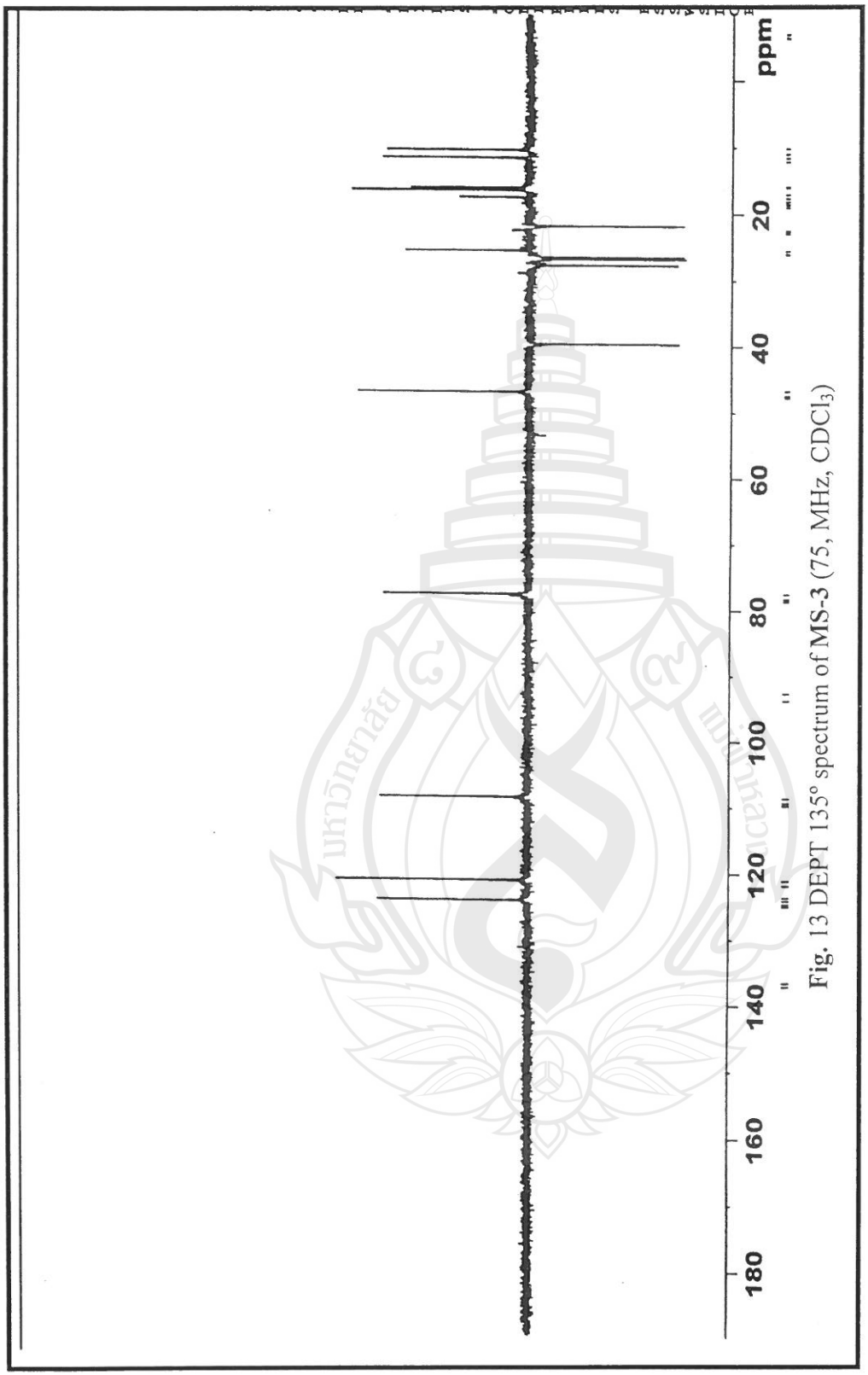

Fig. 13 DEPT 135° spectrum of **MS-3** (75, MHz, $CDCl_3$)

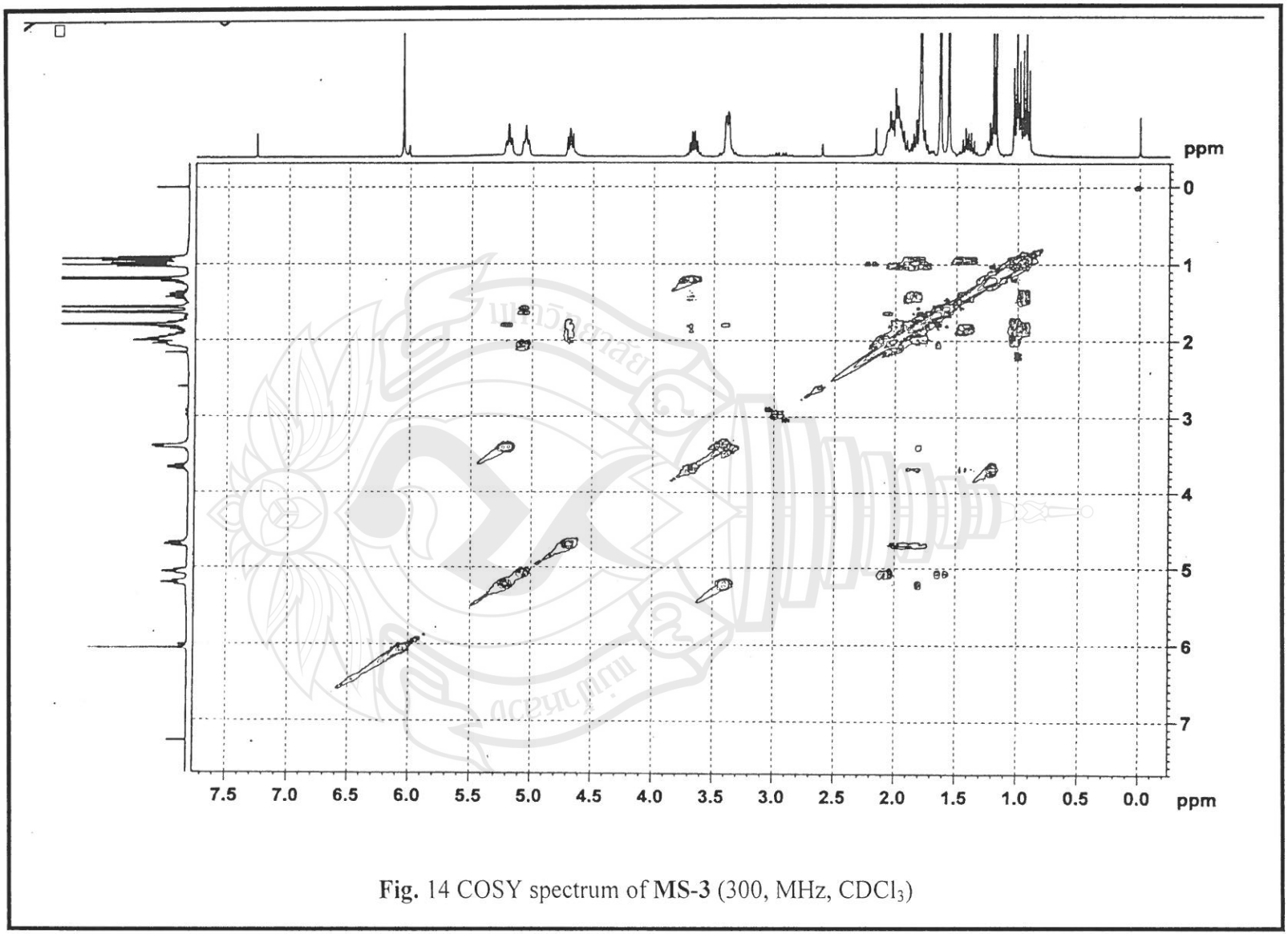

Fig. 14 COSY spectrum of **MS-3** (300, MHz, $CDCl_3$)

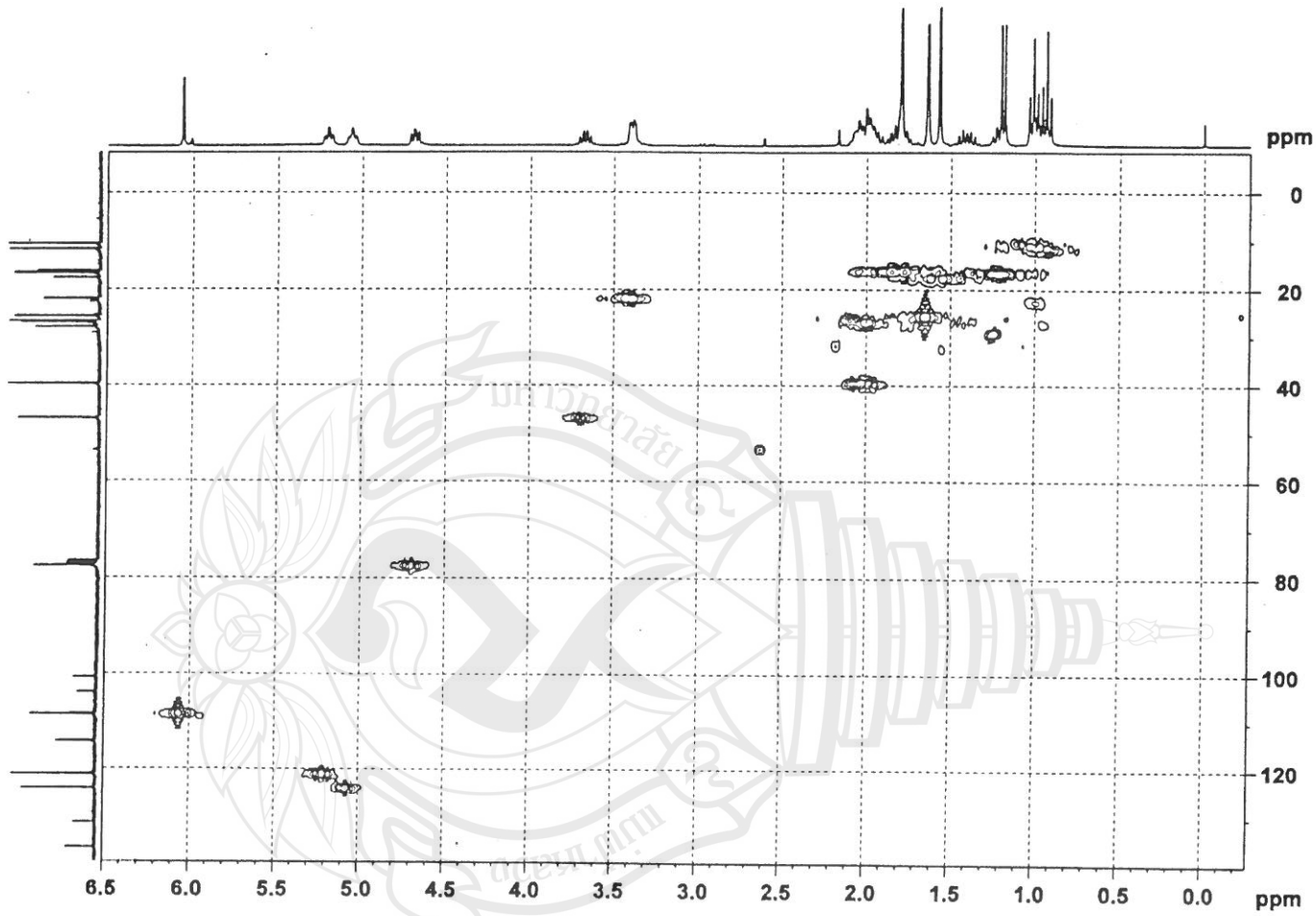

Fig. 15 HMQC spectrum of **MS-3** (300, MHz, $CDCl_3$)

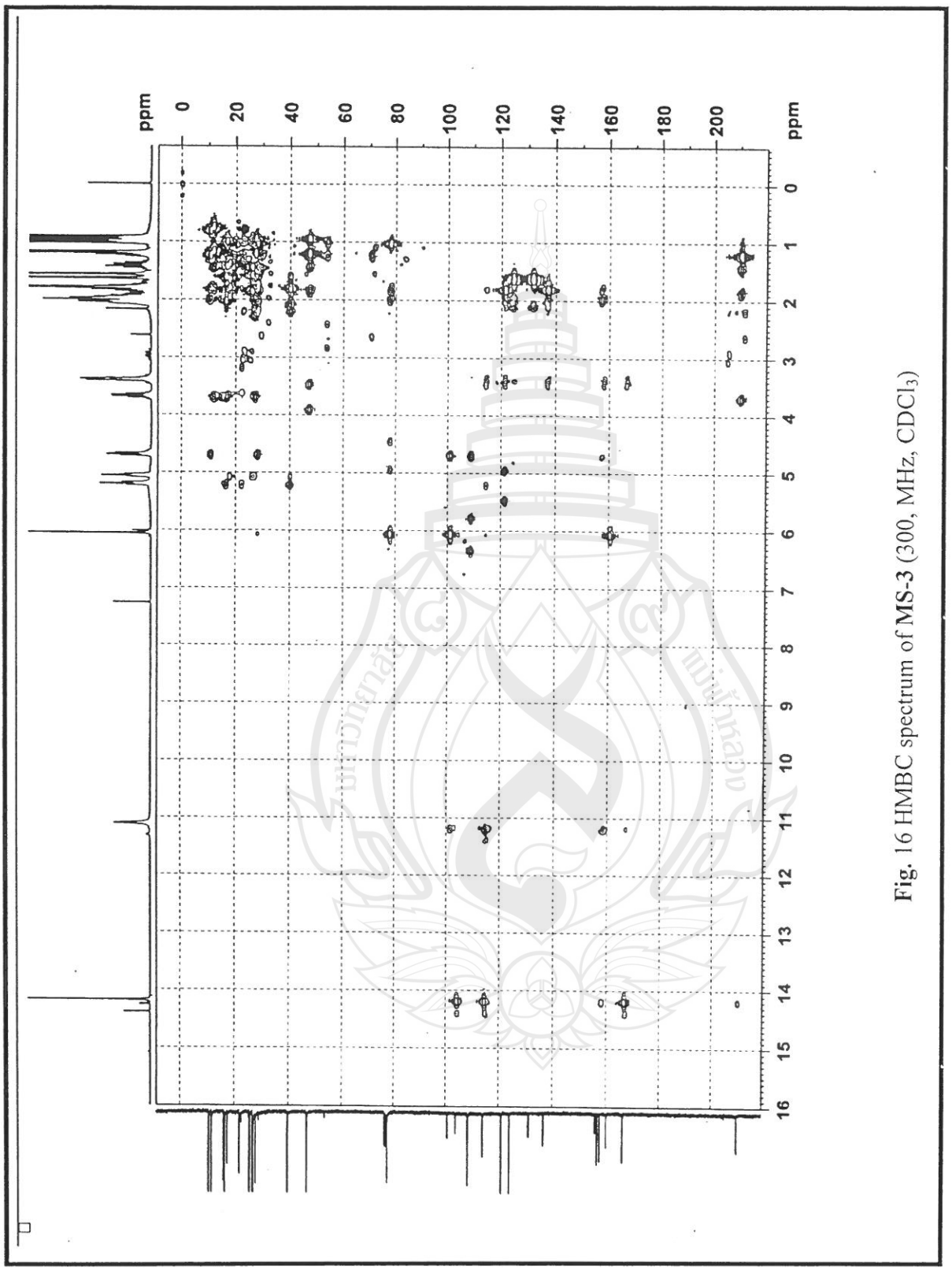

Fig. 16 HMBC spectrum of **MS-3** (300, MHz, $CDCl_3$)

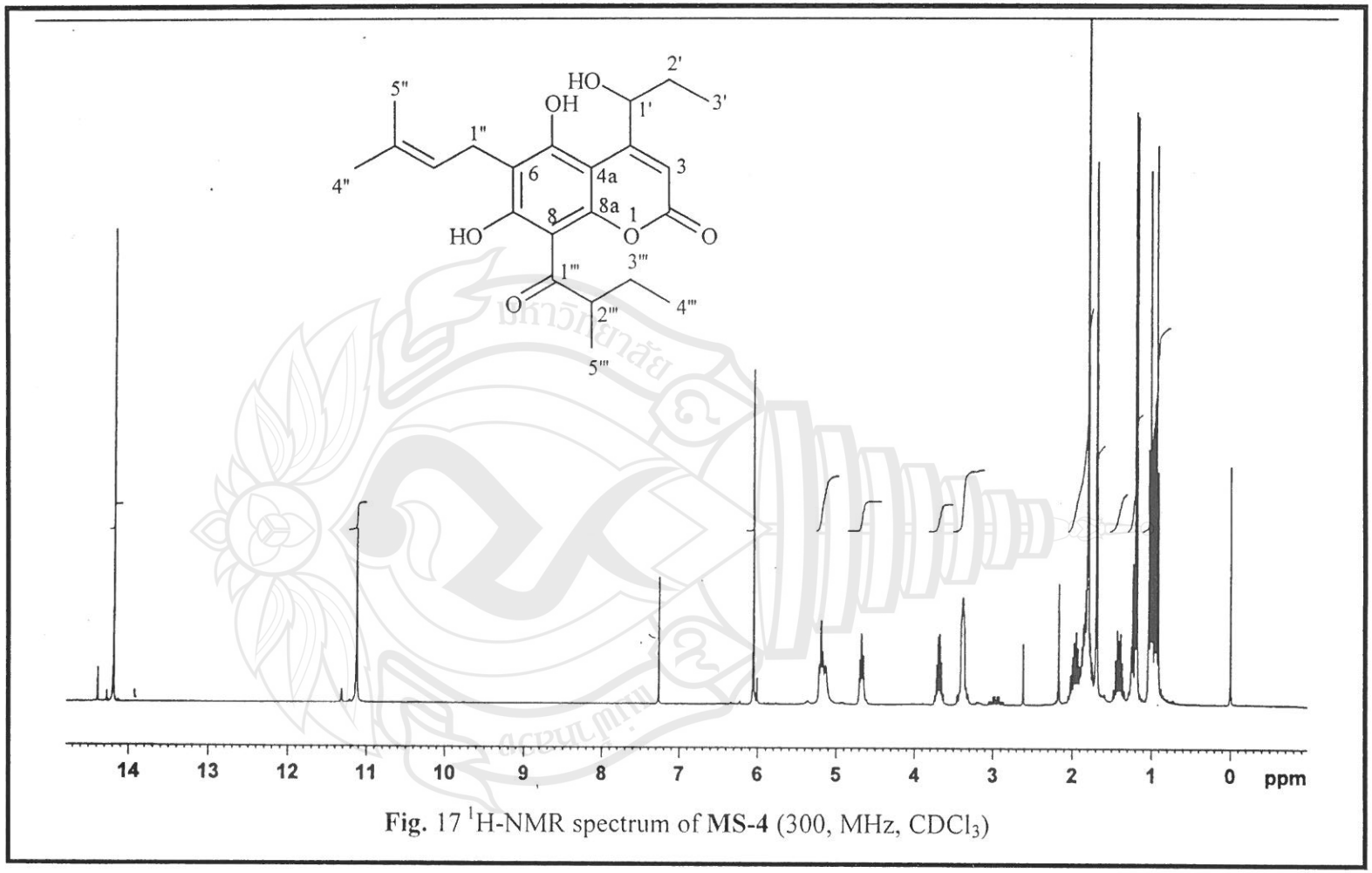

Fig. 17 ^{1}H-NMR spectrum of **MS-4** (300, MHz, $CDCl_3$)

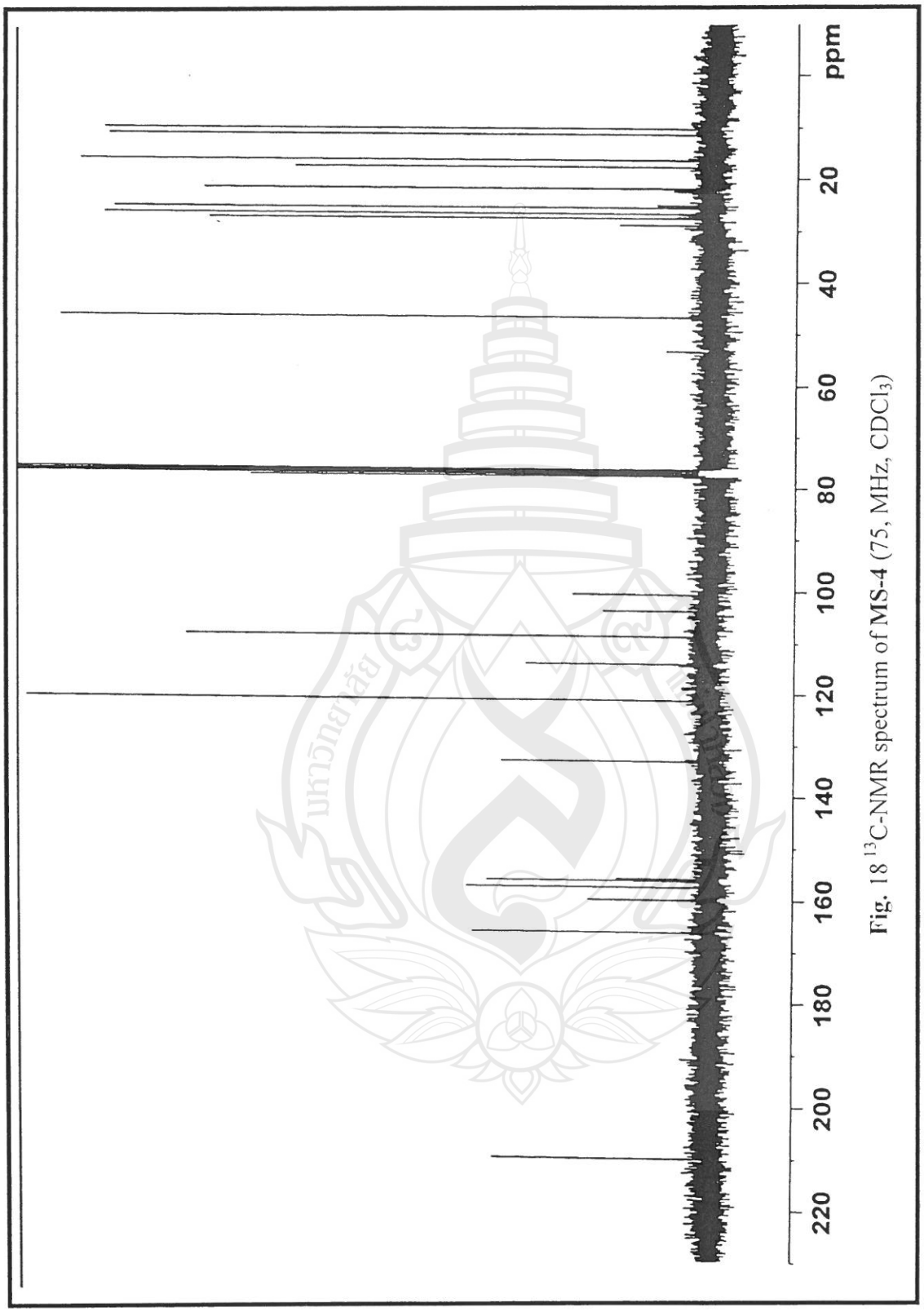

Fig. 18 ^{13}C-NMR spectrum of **MS-4** (75, MHz, $CDCl_3$)

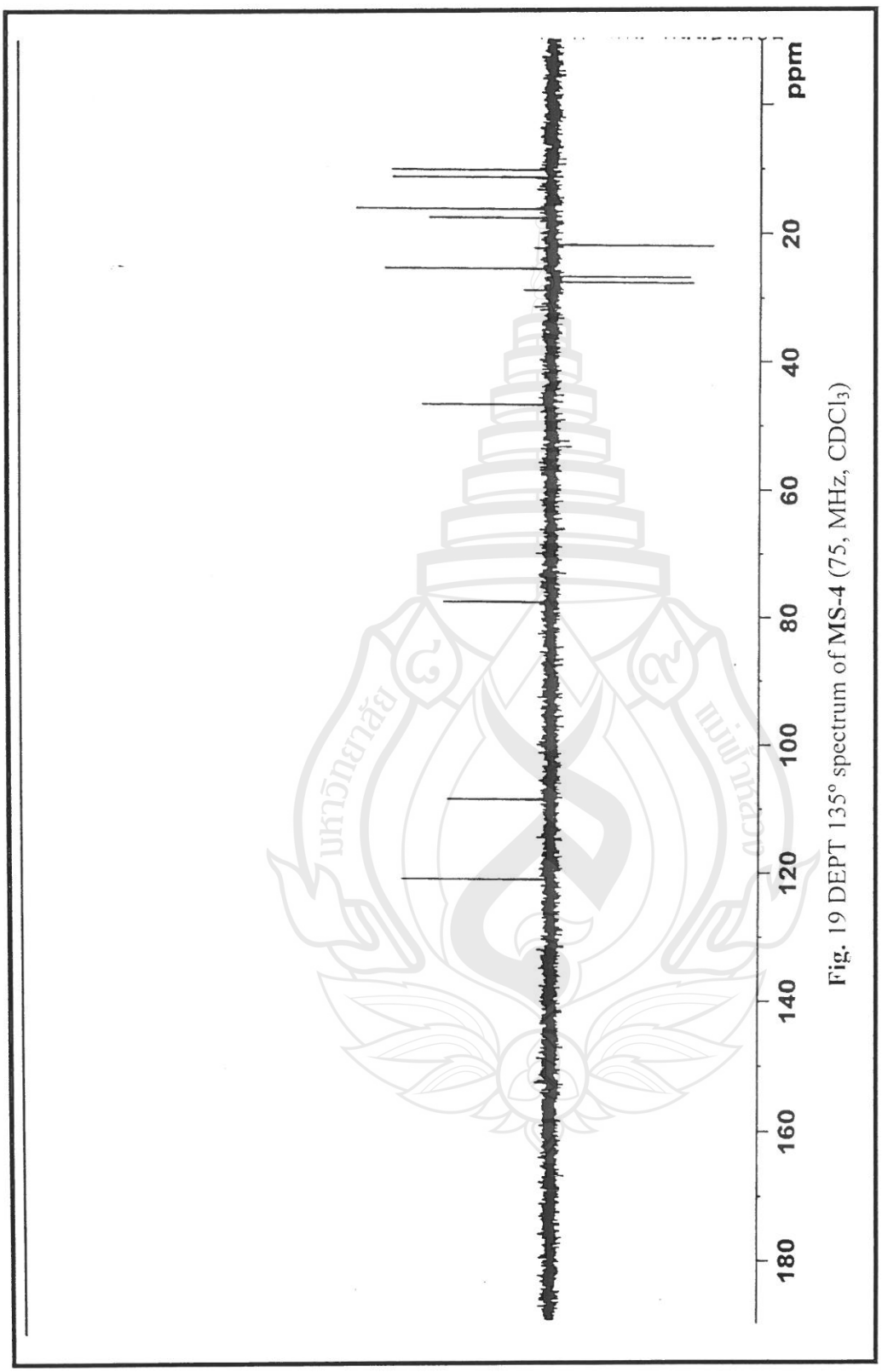

Fig. 19 DEPT 135° spectrum of **MS-4** (75, MHz, $CDCl_3$)

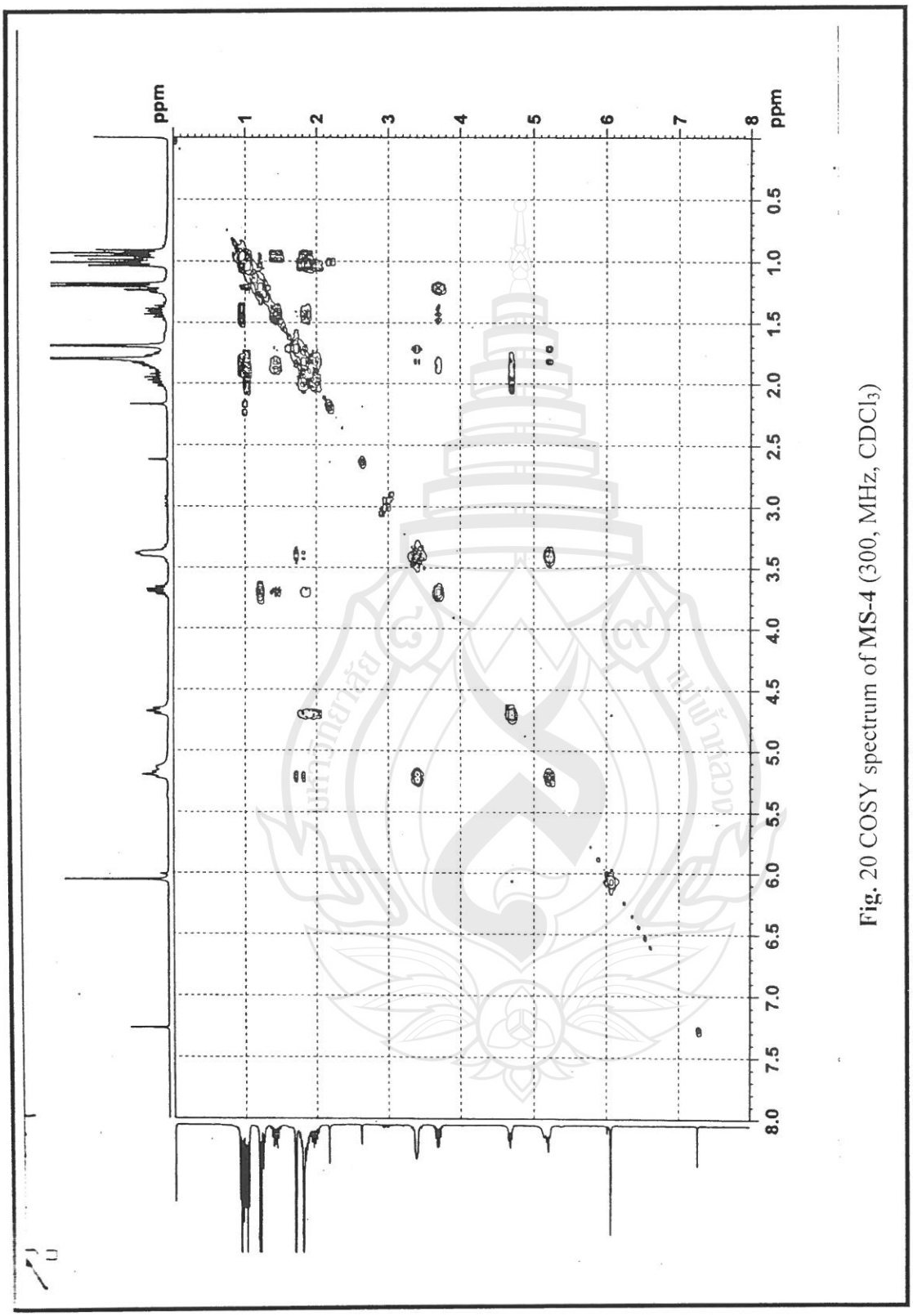

Fig. 20 COSY spectrum of **MS-4** (300, MHz, $CDCl_3$)

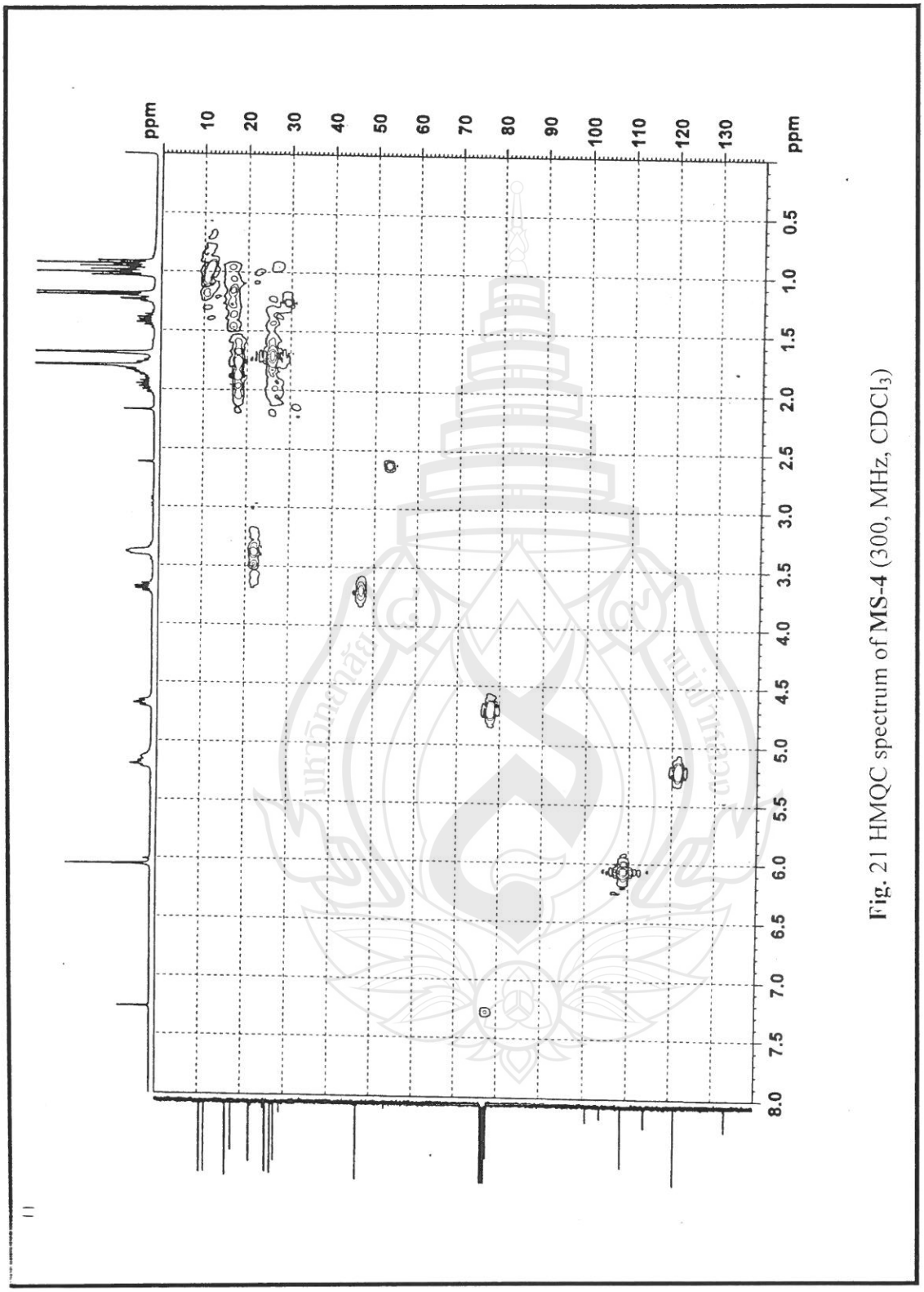

Fig. 21 HMQC spectrum of **MS-4** (300, MHz, $CDCl_3$)

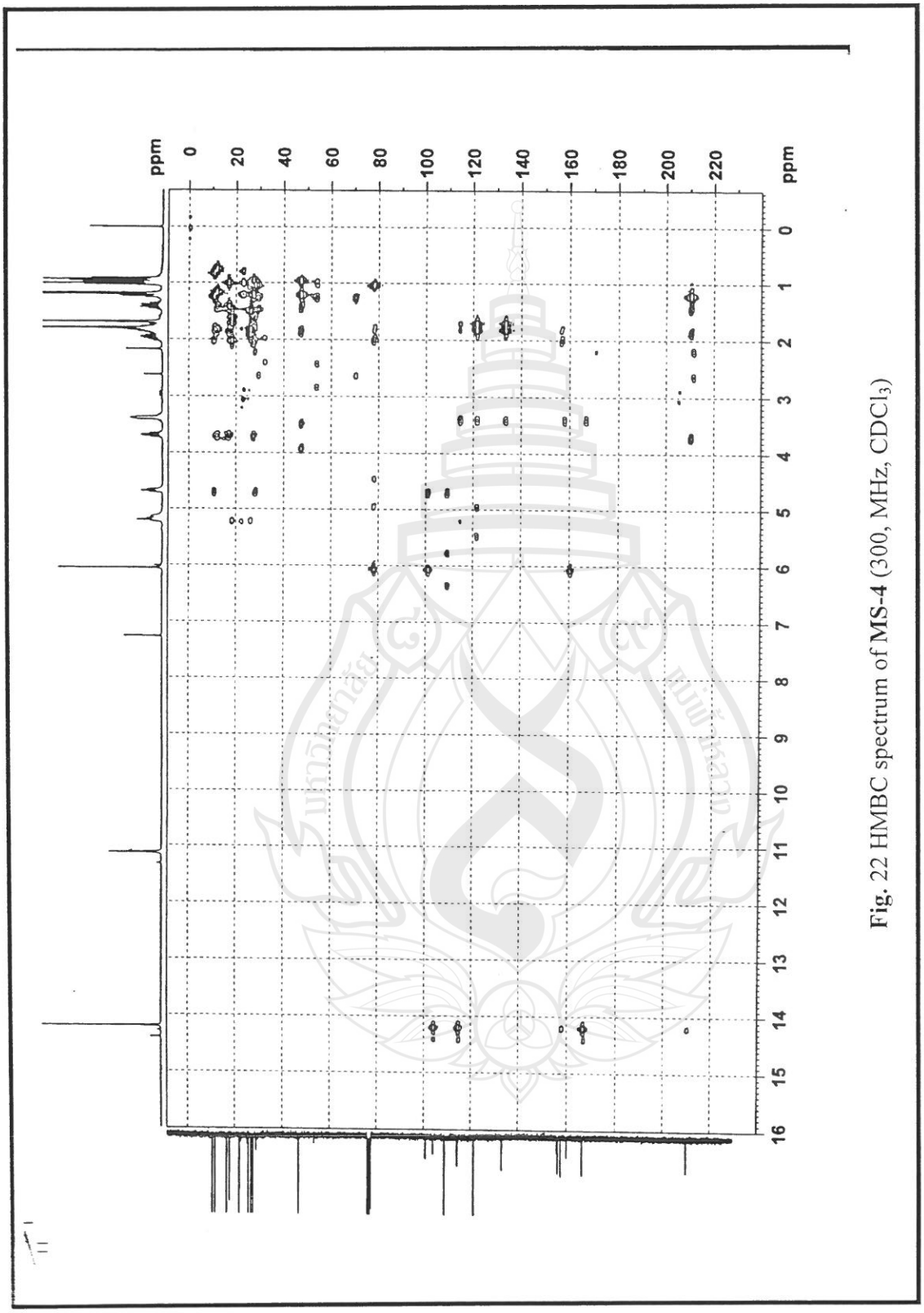

Fig. 22 HMBC spectrum of **MS-4** (300, MHz, $CDCl_3$)

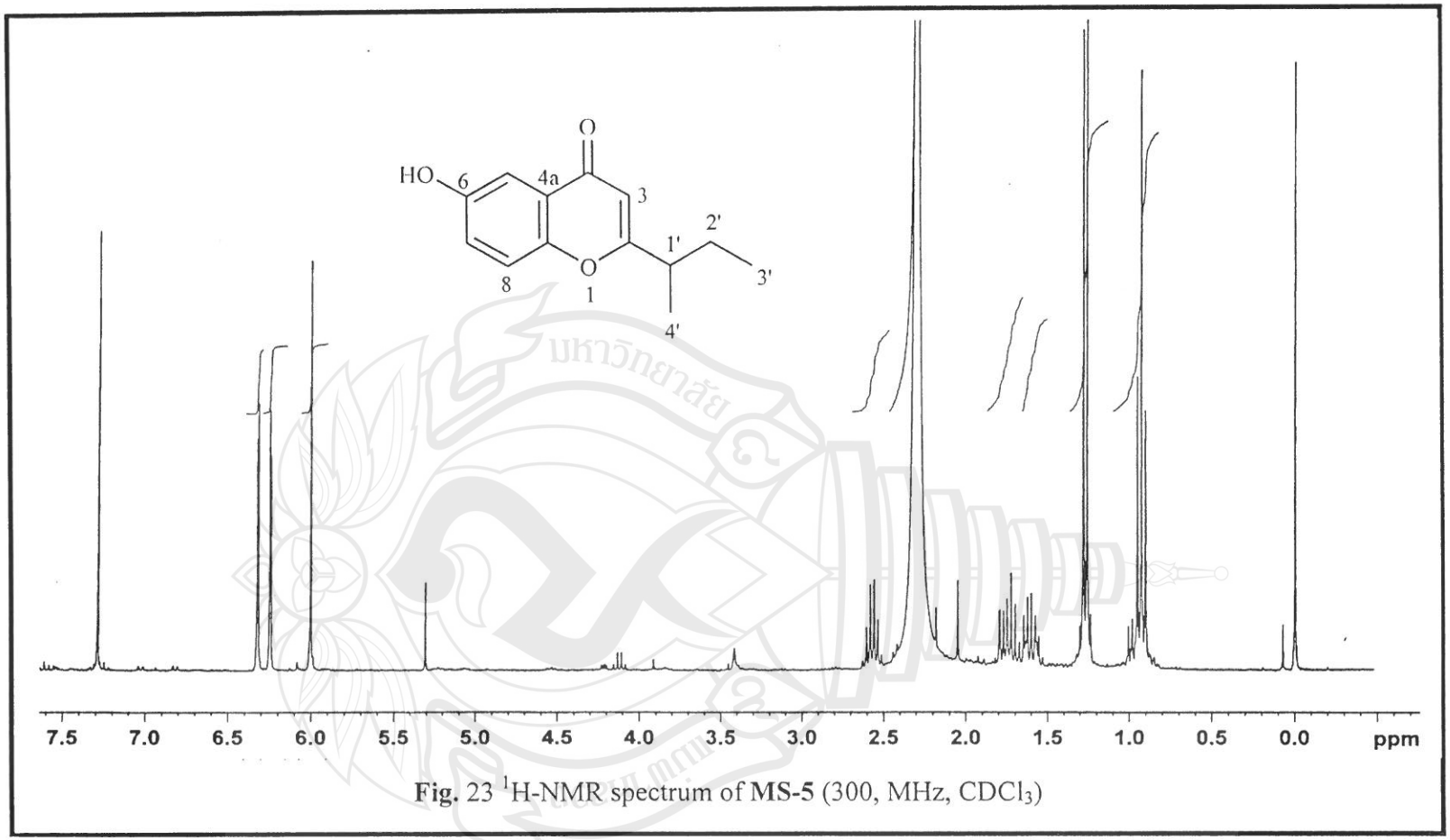

Fig. 23 ^{1}H-NMR spectrum of **MS-5** (300, MHz, $CDCl_3$)

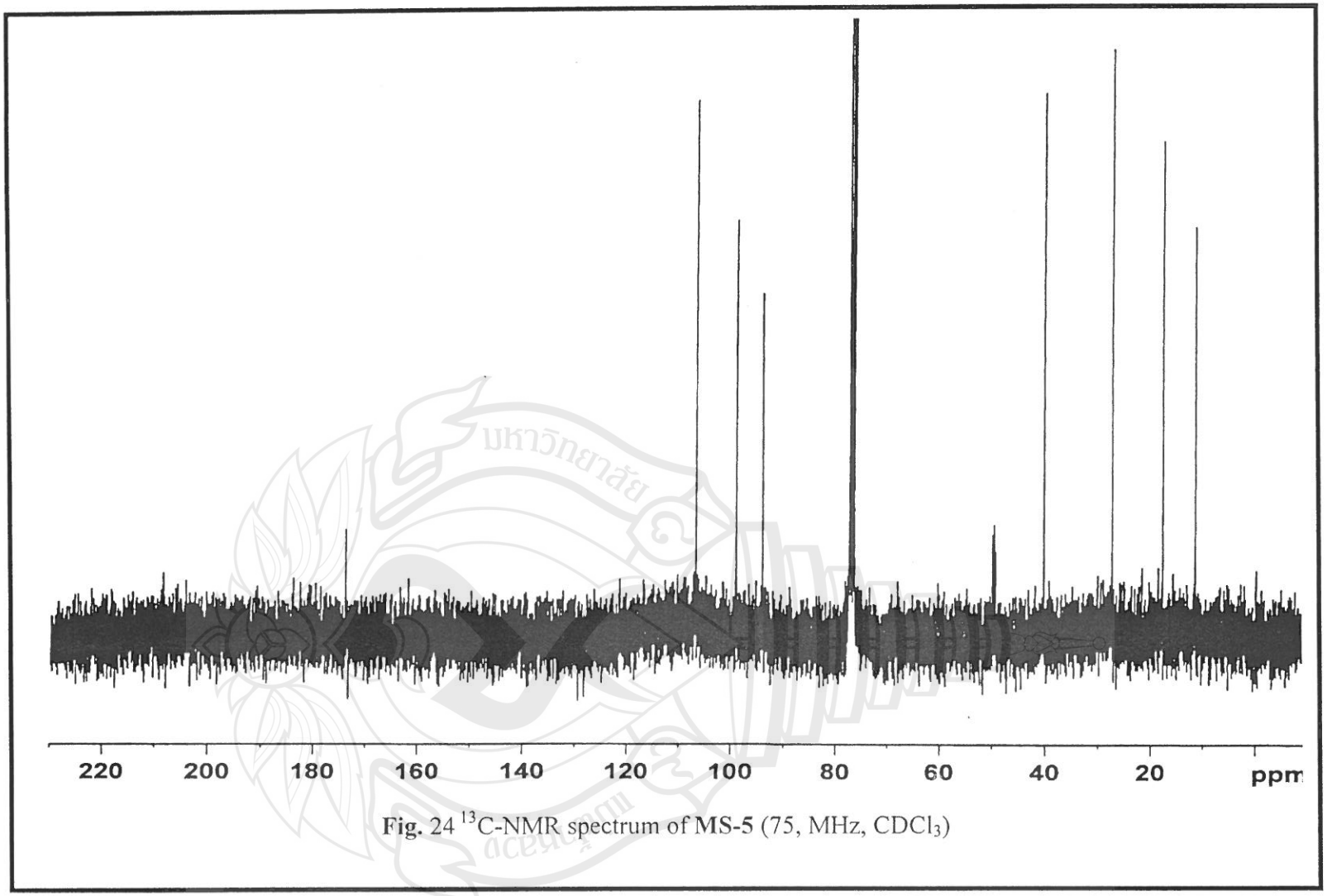

Fig. 24 ^{13}C-NMR spectrum of **MS-5** (75, MHz, $CDCl_3$)

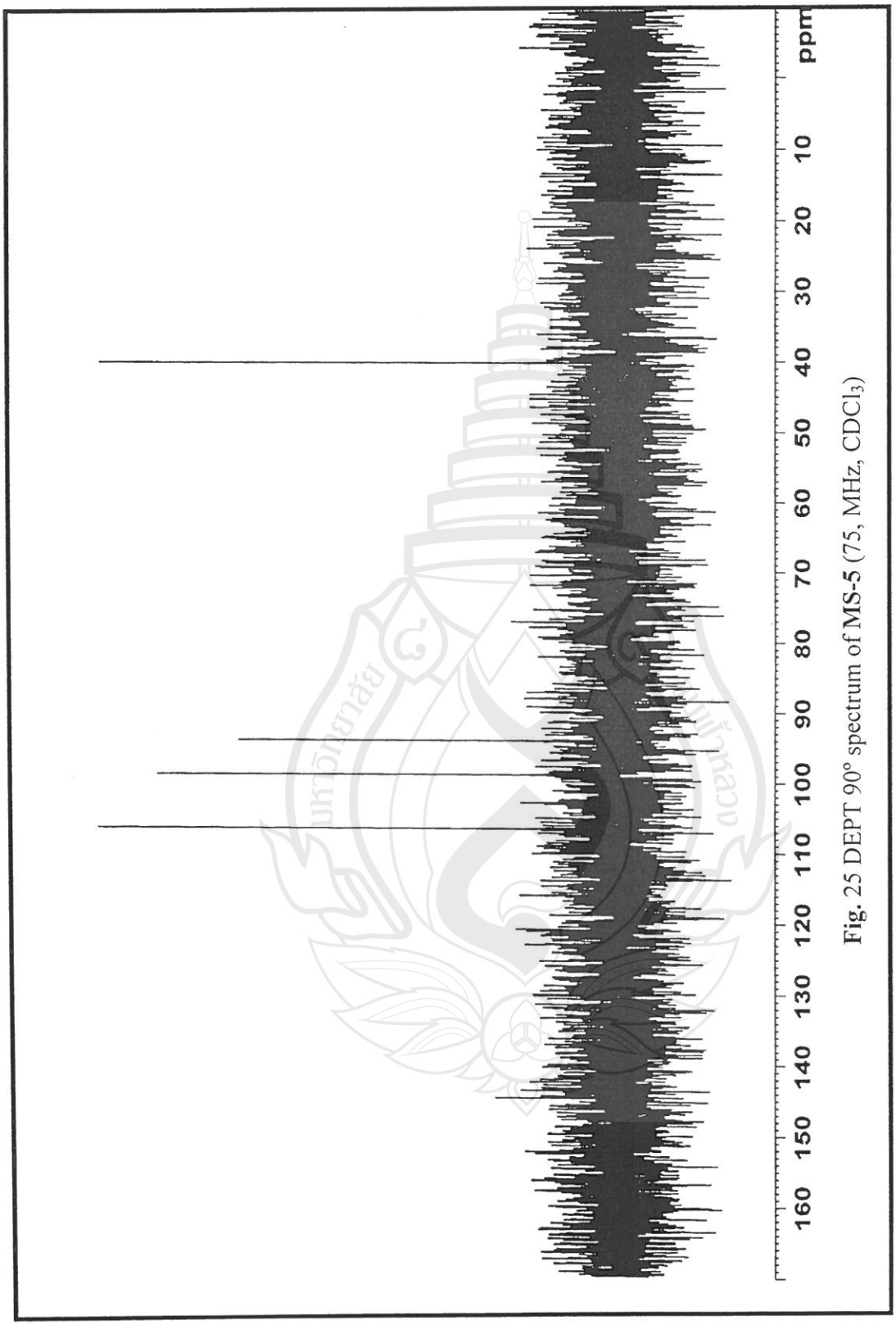

Fig. 25 DEPT 90° spectrum of **MS-5** (75, MHz, $CDCl_3$)

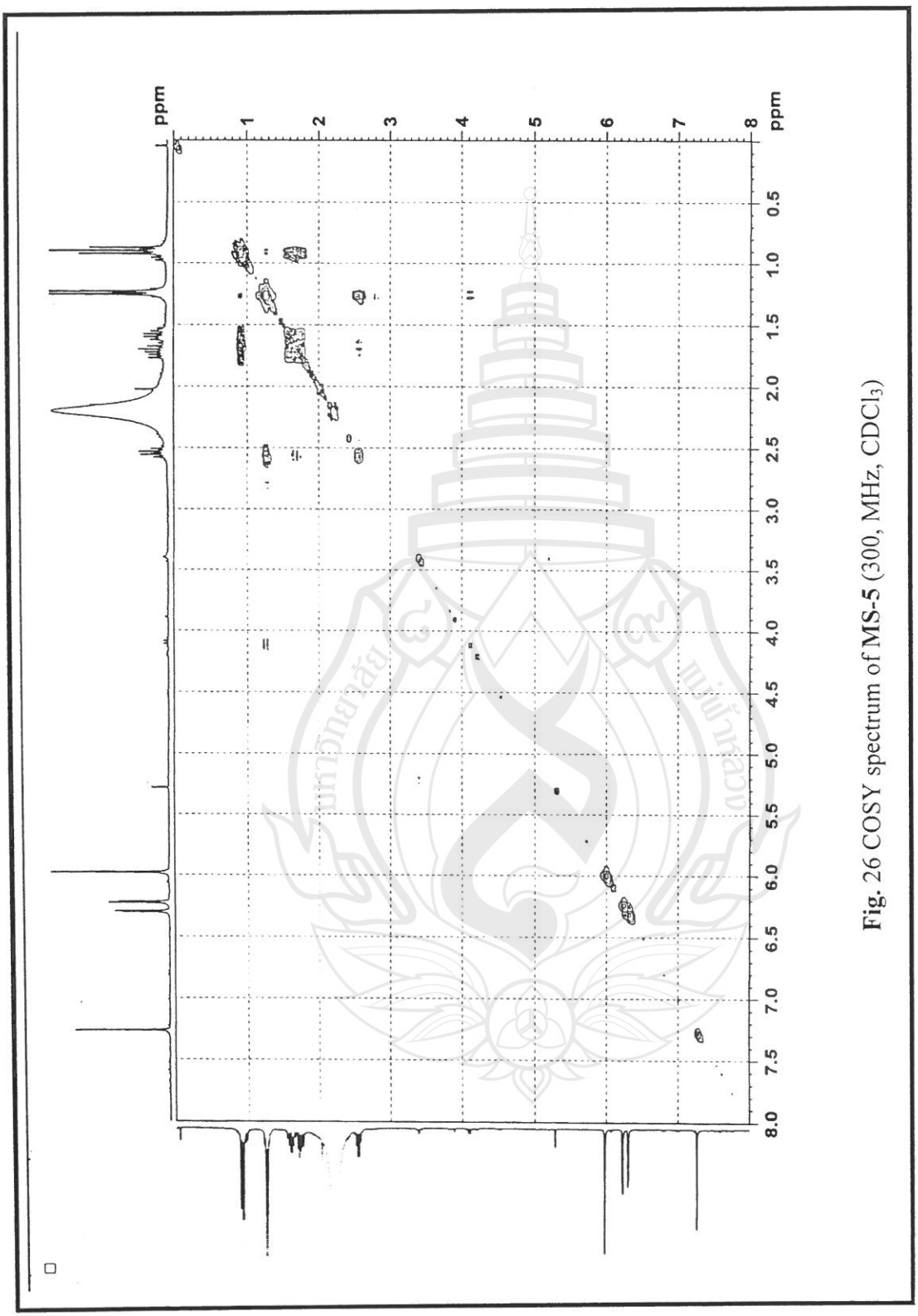

Fig. 26 COSY spectrum of **MS-5** (300, MHz, $CDCl_3$)

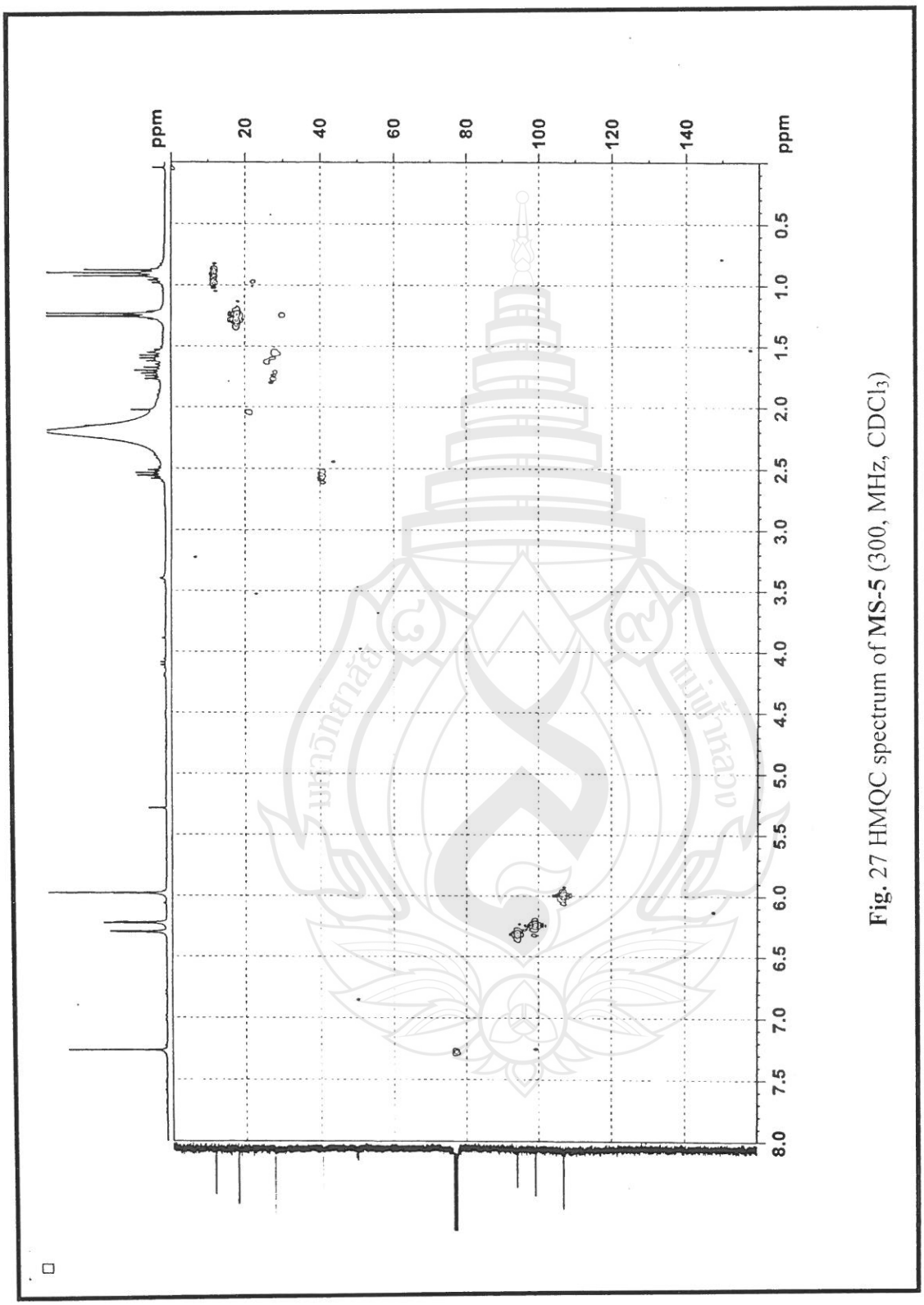

Fig. 27 HMQC spectrum of **MS-5** (300, MHz, $CDCl_3$)

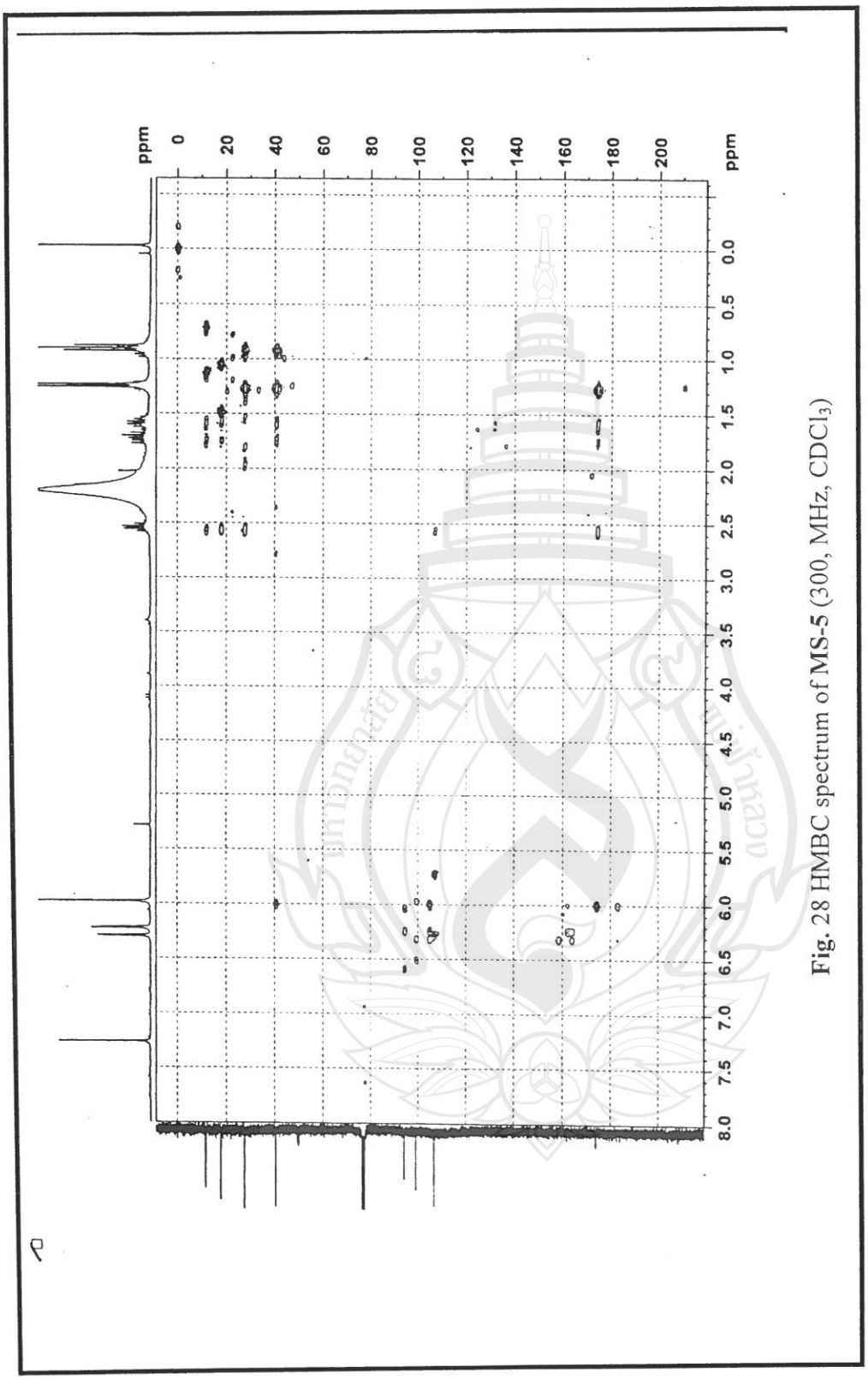

Fig. 28 HMBC spectrum of **MS-5** (300, MHz, $CDCl_3$)

BIOGRAPY

1. Dr. Surat Laphookhieo

School of Science, Mae Fah Luang University

Education background

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

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10. Karalai, C. and **Laphookhieo, S.** "Triterpenoid esters from *Bruguira cylindrica*" *Aust. J. Chem.*, 2005, **58**, 556-559.

11. **Laphookhieo, S.**; Karalai, C.; Ponglimanont, C. "New sesquiterpenoid and triterpenoids from the fruits of *Rhizophora mucronata*" *Chem. Pharm. Bull.*, 2004, **52**, 883-885.

12. **Laphookhieo, S.**; Karalai, C.; Ponglimanont, C.; Chantrapromma, K. "Pentacyclic triterpenoid esters from the fruits of *Bruguira cylindrica*" *J. Nat. Prod.*, 2004, **67**, 886-888.

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Publications

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1. Laphookhieo, S.; Promnart, P.; Syers. J. K.; Kanjana-O-pas, A.; Ponglimanont, C.; Karalai, C. "Coumarins and xanthones from the seed of *Mammea siamensis*" *J. Braz. Chem. Soc.*, 2007, **18**, 1077-1080.

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Phenolic compounds from *Mammea siamensis* seeds

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Abstract: The investigation of dichloromethane and acetone extracts of the seeds of *Mammea siamensis* led to the isolation of a novel phenolic compound, siamensone A (**1**), together with three known compounds, suragin B (**2**), mammea E/BB (**3**), and δ-tocotrienol (**4**). The structures of the isolates were characterized by spectroscopic methods, and all compounds were reported for the first time as metabolites of *M. siamensis*.

Key words: Mammea siamensis, siamensone, coumarins.

Résumé : Une étude des produits d'extraction des graines de *Mammea siamensis* par le dichlorométhane et l'acétone a permis d'isoler un nouveau produit phénolique, la siamensone A (**1**), aux côtés de trois composés déjà connus, la suragine B (**2**), la mammea E/BB (**3**) et le δ-tocotriénol (**4**). Les structures des composés isolés ont été déterminées par des méthodes spectroscopiques et tous les composés ont été caractérisés pour la première fois comme métabolites de *M. siamensis*.

Mots clés : Mammea siamensis, siamensone, coumarines.

[Traduit par la Rédaction]

Introduction

Mammea belongs to the family of Guttiferae, typically found in several southeast asian countries. Two species are found in Thailand, *M. siamensis* (syn. *Ochrocarpus siamensis*) and *M. harmandii* (1). The flowers of *M. siamensis* have been used as a heart tonic in local medicine. A number of secondary metabolites have been isolated from both species (2–8). Our previous phytochemical studies of Thai medicinal plants led to the isolation and identification of xanthones (9), triterpenoids (10–12), cardenolide glycosides (13), and diterpenes (14). As a continuation of our studies on Thai medicinal plants, we now report the isolation of a novel chromone, siamensone A (**1**) and three other known compounds (**2**–**4**) from the seeds of *M. siamensis*. In addition, the ^{13}C NMR spectral data of **2** and **3** is also reported for the first time.

Results and discussion

The dichloromethane and acetones extracts of the seeds of *M. siamensis* were subjected to column chromatography to give a new compound (**1**) together with three other known compounds (**2**–**4**) shown in Chart 1. Their structures were elucidated using 1D and 2D NMR spectroscopic data and compared with those reported in the literature.

Siamensone A (**1**), yellowish solid, is a 6,8-dihydroxy-2-*sec*-butyl-4*H*-chromen-4-one. Its molecular formula of $C_{13}H_{14}O_4$ with a molecular ion $[M]^+$ at *m/z* 234.0877 (calc. $C_{13}H_{14}O_4$ *m/z* 234.0892) was established by HR-EIMS analysis. This compound exhibited UV absorption maxima at 225, 248, 300, and 337 nm, suggesting the presence of conjugation in the molecule. The IR spectrum showed absorption bands of OH stretching at 3399 cm^{-1} and C=O stretching at 1714 cm^{-1}. The ^{13}C NMR spectrum also showed the resonance of a carbonyl carbon at δ: 182.7 (C-4). The ^1H NMR spectrum (Table 1) displayed the characteristic signals of *meta*-coupled aromatic protons at δ: 6.32 (d, J = 2.1 Hz, H-5) and 6.24 (d, J = 2.1 Hz, H-7) and a singlet signal of an olefinic proton at δ: 6.00 (*s*, H-3). With combination of the COSY spectrum, a *sec*-butyl moiety was evident from ^1H NMR signals at δ: 2.58 (sextet, J = 6.9 Hz, H-1′), 1.70–1.80 (m, H-2′a), 1.55–1.65 (m, H-2′b), 1.27 (d, J = 6.9 Hz, Me-4′), and 0.93 (t, J = 7.5 Hz, Me-3′). The *sec*-butyl moiety was located at C-2 position based on Heteronuclear Multiple Bond Correlations (HMBC) (Table 1). The singlet methine proton signal at δ: 6.00 (H-3) correlated to C-1′ (40.4), and a sextet methine proton at δ: 2.58 (H-1′) correlated to C-2 (174.2) and C-3 (106.7). The other HMBC correlations were summarized in Table 1. Therefore, compound **1** was deduced to be siamensone A.

The remaining compounds were characterized as suragin B (**2**) (15, 16), mammea E/BB (**3**) (16), and δ-tocotrienol (**4**) (17) by the analysis of 1D and 2D NMR data and by comparison with their reported physical and spectroscopic data. In addition, the complete assignments of ^{13}C NMR of

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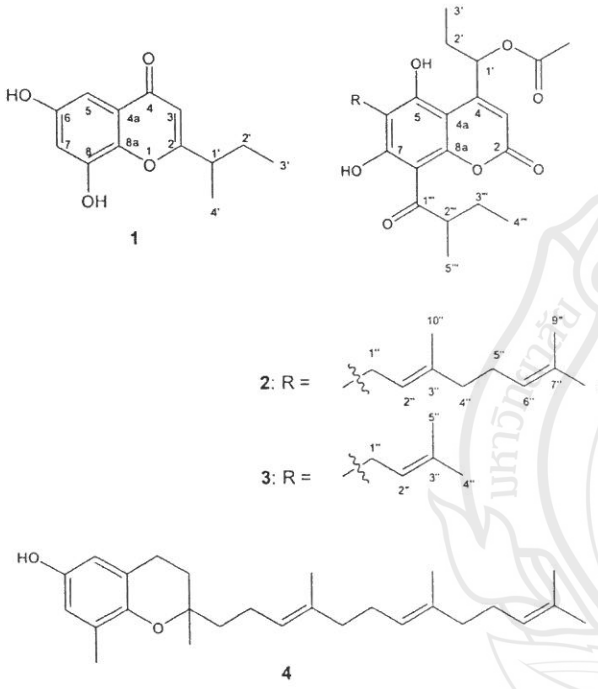
Table 1. ^1H (300 MHz), ^{13}C NMR (75 MHz), COSY, and HMBC spectral data of siamensone A (**1**) in $CDCl_3$.

C/H	δ_C	δ_H (J in Hz)	^1H–^1H COSY	HMBC Correlations $^1H \rightarrow ^{13}C$
2	174.2			
3	106.7	6.00 (s)		C-2, C-4, C-4a, C-1′
4	182.7			
4a	105.0			
5	94.2	6.32 (d, J = 2.1)	H-7	C-4, C-4a, C-6, C-7, C-8a
6	163.2			
7	99.0	6.24 (d, J = 2.1)	H-5	C-5, C-6, C-8, C-8a
8	161.8			
8a	158.3			
1′	40.4	2.58 (sextet, J = 6.9)	H-2′, H-4′	C-2, C-3, C-2′, C-3′, C-4′
2′	27.5	1.70–1.80 (m)	H-1′, H-3′	C-2, C-1′, C-3′, C-4′
		1.55–1.65 (m)		
3′	11.5	0.93 (t, J = 7.5)	H-2′	C-1′, C-2′
4′	17.7	1.27 (d, J = 6.9)	H-1′	C-1′, C-2′

Chart 1.



suragin B (**2**) and mammea E/BB (**3**) are also reported here for the first time (Table 2).

Experimental section

General experimental procedures

Melting points were determined using a Fisher–Johns melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. UV spectra were measured with a UV-160A spectrophotometers (Shimadzu, Kyoto, Japan). The IR spectra were measured with a PerkinElmer FTS FTIR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded using 400 and/or 300 MHz Bruker FTNMR Ultra Shield spectrometers. Chemical shifts were recorded in ppm (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) (18, 19) and column chromatography (CC) were carried out on silica gel 60 H (Merck, Rahway, NJ; 5–40 μm) and silica gel 100 (Merck, Rahway, NJ; 63–200 μm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Plant material

The seeds of *M. siamensis* were collected from Mae Fah Luang University, Tasud, Muang, Chiang Rai province, northern part of Thailand in August 2005. Plant identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, and the voucher specimen (No. SC09) was deposited at Prince of Songkla University Herbarium.

Extraction and isolation

The seeds (224.5 g) of *M. siamensis* were extracted with dichloromethane and acetone, respectively, at room temperature (each for 5 days) and evaporated under reduced pressure to provide dichloromethane extract (44.4 g) and acetone extract (30.5 g). The dichloromethane extract (44.4 g) was chromatographed by QCC (column size: 12 × 16 cm) and eluted with EtOAc–hexane mixtures to give seven fractions (F1–F7). Fraction F4 (3.35 g) was separated by CC with EtOAc–hexane (3:17) followed by RP-18 preparative TLC with MeOH–H_2O (4:1) to afford five subfractions (F4a–F4e). Subfraction F4d (1.02 g) was purified by RP-18 CC with MeOH–H_2O (4:1) followed by CC with EtOAc–hexane (1:3) to afford compound **1** (12.7 mg).

The acetone extract (30.5 g) was chromatographed by QCC (column size: 12 × 16 cm) and eluted with hexane–acetone (5:1) to give seventeen fractions (F1–F17), of which fractions F8–F10 (1.5 g) gave compound **2**. Fraction F2 (4 g) was subjected to repeated QCC with hexane–acetone mixtures (95% hexane–acetone to 100% acetone) to afford compound **2** (104 mg) and 5 subfractions (F2-1–F2-5). A portion (450 mg) of subfraction F2-2 (1.05 g) was subjected to repeated CC with 10% EtOAc–hexane to afford 14 subfractions (F2-2A–F2-2N). Compound **4** (7.7 mg)

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **2** and **3** in $CDCl_3$.

	2		**3**	
C/H	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	159.3		159.7	
3	106.3	6.28 (s)	106.3	6.28 (s)
4	157.2	—	155.7	—
4a	100.3	—	100.3	—
5	158.2	—	158.3	—
6	110.2	—	110.1	—
7	165.5	—	165.9	—
8	104.6	—	104.3	—
8a	156.0	—	156.5	—
1′	73.7	6.49 (dd, J = 2.1, 8.0)	73.7	6.50 (dd, J = 2.7, 8.1)
2′	27.1	1.88–1.98 (m)	27.1	1.89–2.01 (m)
3′	10.1	1.00 (t, J = 7.2)	10.1	1.01 (t, J = 7.5)
1″	21.0	3.48 (dd, J = 4.4, 16.5)	21.6	3.49 (dd, J = 7.6, 16.6)
		3.52 (dd, J = 4.4, 16.5)		3.55 (dd, J = 6.4, 16.6)
2″	123.2	5.24 (t, J = 7.1)	119.1	5.25 (t, J = 7.0)
3″	142.4	—	138.9	—
4″	40.4	2.08–2.18 (m)	18.0	1.81 (s)
5″	26.3	2.08–2.18 (m)	25.9	1.87 (s)
6″	119.7	5.04–5.11 (m)		—
7″	132.2	—		—
8″	18.8	1.67 (s)		—
9″	25.5	1.59 (s)		—
10″	16.4	1.87 (s)		—
1‴	210.7	—	210.7	—
2‴	46.5	3.91 (sextet, J = 7.0)	47.0	3.80 (sextet, J = 6.6)
3‴	28.7	1.42–1.50 (m)	28.7	1.40–1.46 (m)
		1.81–1.87 (m)		1.83–1.88 (m)
4‴	11.7	0.98 (t, J = 7.4)	11.7	0.98 (t, J = 7.5)
5‴	16.6	1.24 (d, J = 7.0)	16.6	1.26 (d, J = 6.6)
CH_3CO	21.0	2.19 (s)	21.0	2.19 (s)
CH_3CO	170.3	—	170.5	—
5–OH		7.21 (s)		7.18 (s)
7–OH		14.64 (s)		14.64 (s)

tained from subfraction F2-2G by repeated CC with 90% hexane–acetone. Repeated CC of subfraction F2-4 with 10% EtOAc–hexane gave compound **3** (11.0 mg).

Siamensone A (6,8-dihydroxy-2-*sec*-butyl-4*H*-chromen-4-one)

Yellowish solid; mp 177–178 °C. $[\alpha]^{27}_D$ −25.0° (*c* 1.35, MeOH). UV (MeOH) λ_{max} (log ε): 225 (4.14), 248 (3.92), 300 (3.42), 337 (3.41). IR (neat): 3399, 1714, 1609. ^{1}H and ^{13}C NMR: see Table 1. EIMS *m/z* (%): 234 ([M$^+$], 90), 233 (100), 177 (19), 176 (23). HR-EIMS *m/z*: [M$^+$] calcd. for $C_{13}H_{14}O_4$, 234.0892; found, 234.0877.

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Short Report

Coumarins and Xanthones from the Seeds of *Mammea siamensis*

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Uma cumarina inédita, mammea E/BB ciclo D (**1**), juntamente com cinco compostos conhecidos, mammea E/BA ciclo D (**2**), suragina C (**3**), terapina B (**4**), 1,7-dihidroxixantona (**5**) e 1-hidróxi-5-metoxyxantona (**6**), foram isolados de sementes de *Mammea siamensis*. Suas estruturas foram caracterizadas usando dados de RMN 1D e 2D. Suragina C e terapina B mostraram atividade citotóxica contra adenocarcinoma de mama (MCF-7), câncer cervical humano (HeLa), câncer de colon (HT-29) e câncer oral humano (KB).

A new coumarin, mammea E/BB cyclo D (**1**), together with five known compounds, mammea E/BA cyclo D (**2**), suragin C (**3**), therapin B (**4**), 1,7-dihydroxyxanthone (**5**) and 1-hydroxy-5-methoxyxanthone (**6**), were isolated from the seeds of *Mammea siamensis*. Their structures were characterized using 1D and 2D NMR spectral data. Suragin C and therapin B showed cytotoxic activity against breast adenocarcinoma (MCF-7), human cervical cancer (HeLa), colon cancer (HT-29) and human oral cancer (KB).

Keywords: mammea E/BB cyclo D, cytotoxic activity, *Mammea siamensis*, guttiferrae

Introduction

Mammea siamensis (Miq) T. Anders. (Guttiferae), known in Thai as "Sarapi", is a small evergreen tree distributed in Thailand, Laos, Cambodia, Vietnam and Myanmar. The flowers of this plant have been used in traditional Thai medicine as a heart tonic. Investigations of different parts of the plant have revealed the presence of several coumarins and xanthones.[1-4] We have previously reported the isolation and structure determination of phenolic compounds from the seeds of this species.[5] In a continuation of our study on this plant, we now report herein the isolation and structure elucidation of a novel compound, mammea E/BB cyclo D (**1**), together with three known coumarins, mammea E/BC cyclo D (**2**),[3] suragin C (**3**),[6] therapin B (**4**)[7] and two known xanthones, 1,7-dihydroxyxanthone (**5**)[8] and 1-hydroxy-5-methoxyxanthone (**6**)[9] from the CH_2Cl_2 extract (Figure 1). The cytotoxic activity of all isolates is also reported.

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Experimental

General procedures

Melting points were determined using a Fisher-John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with a JASCO P-1020 polarimeter. UV spectra were measured with a UV-160A spectrophotometer (Shimadzu). The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded using 500 MHz Varian UNITY INOVA and 300 MHz Bruker FTNMR Ultra Shield™ spectrometers. Chemical shifts were recorded in parts *per* million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The EIMS was obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck, 230-400 Mesh ASTM) and silica gel 100 (Merck, 70-230 Mesh ASTM), respectively. Precoated plates of silica gel 60 F_{254} and reversed-phase (RP-18 F_{254S}) were used for analytical purposes.

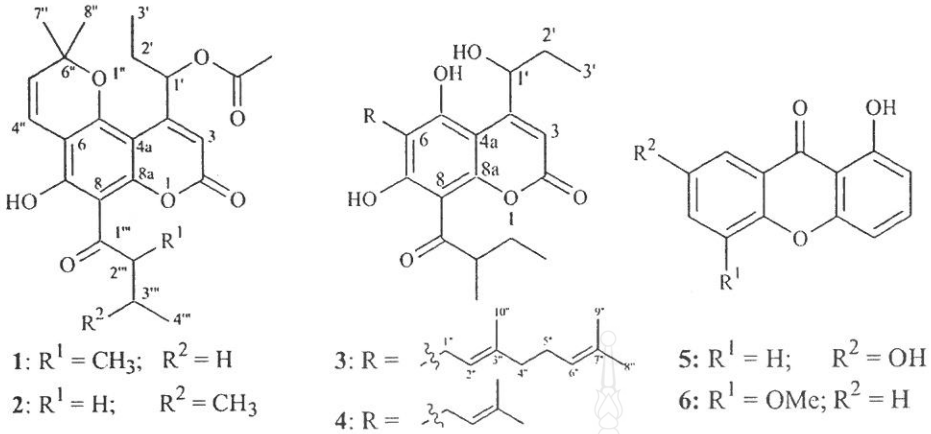

Figure 1. Structures of compounds **1-6**.

Plant material

The seeds of *M. siamensis* were collected from Mae Fah Luang University, Tasud, Muang, Chiang Rai Province, northern Thailand in August 2005. The identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a voucher specimen (No. SC09) was deposited at Prince of Songkla University Herbarium.

Extraction and Isolation

The seeds (224.5 g) of *M. siamensis* were extracted successively with CH_2Cl_2 (500 mL) at room temperature for 5 days. The filtered samples were combined and the solvents were evaporated under reduced pressure to provide the CH_2Cl_2 extracts (44.4 g).

The CH_2Cl_2 extract (44.4 g) was chromatographed by QCC and eluted with hexane-EtOAc mixtures to give seven fractions (F1-F7). Fraction F2 (1.92 g) was purified by RP-18 CC with acetone: H_2O (3:1) and followed by RP-18 preparative TLC with acetone: H_2O (3:1) to yield **1** (3.1 mg) and **2** (4.3 mg). Fraction F4 (3.35 g) was separated by CC with EtOAc: hexane (3:17) and followed by RP-18 preparative TLC with MeOH:H_2O (4:1) to provide five subfractions (F4a-F4e). Subfraction F4b (12.8 mg) was purified by preparative TLC with EtOAc: hexane (1:3, v/v) to give **5** (2.1 mg). Subfraction F4d (1.02 g) was purified by RP-18 CC with MeOH:H_2O (4:1) and followed by CC with EtOAc: hexane (1:3) to afford **4** (16.8 mg) and **3** (32.6 mg). Fraction F6 (167.0 mg) was separated by CC with EtOAc: hexane (2:3, v/v) to give **6** (6.3 mg).

Mammea E/BB cyclo D (1)

Yellowish viscous oil; ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 14.44 (7-OH), 6.74 (1H, d, *J* 10.0 Hz, H-4″), 6.60 (1H, dd, *J* 6.8, 2.8 Hz, H-1′), 6.30 (1H, s, H-3), 5.60 (1H, d, *J* 10.0 Hz, H-5″), 4.02 (1H, sextet, *J* 6.3 Hz, H-2‴), 2.17 (3H, s, H-1′-CO*CH*$_3$), 1.97 (1H, m, H-2′a), 1.80 (1H, m, H-3‴a), 1.78 (1H, m, H-2′b), 1.58 (3H, s, H-7″), 1.56 (3H, s, H-8′), 1.45 (1H, *m*, H-3‴b), 1.26 (3H, d, *J* 6.3 Hz, H-5‴), 1.07 (3H, t, *J* 7.2 Hz, H-3′) and 1.06 (3H, t, *J* 7.2 Hz, H-4‴); ^{13}C NMR data ($CDCl_3$, 75 MHz): 210.8 (C-1‴), 170.3 (*C*H$_3$*C*O), 163.5 (C-7), 159.3 (C-2), 157.5 (C-4), 156.7 (C-8a), 155.7 (C-5), 126.8 (C-5″), 115.8 (C-4″), 106.5 (C-6), 106.4 (C-3), 103.7 (C-8), 100.9 (C-4a), 80.2 (C-6″), 73.0 (C-1′), 46.9 (C-2‴), 29.6 (C-3‴), 28.6 (C-2′), 28.4 (C-7″), 27.8 (C-8″), 21.0 (*C*H$_3$CO), 16.9 (C-5‴), 10.6 (C-4‴), 10.0 (C-3′); EIMS *m/z* (rel. int.): 428 $[M]^+$ (39), 413 (100), 371 (45), 353 (13), 311 (29), 283 (5); HREIMS *m/z* $[M]^+$ 428.1813 (calc. for $C_{24}H_{28}O_7$, 428.1835); UV(MeOH) λ_{max}/nm: 225, 280, 285, 300, 373; IR($CHCl_3$) ν_{max}/cm^{-1}: 3454, 1738, 1655, 1605; $[\alpha]_D^{27}$-15.0° (*c* 0.10, MeOH).

Cytotoxicity assay

The procedure for cytotoxic assay was performed by sulphorhodamine B (SRB) assay as described by Skehan *et al.*[10] In this study, four cancer cell lines, MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) were used. Camptothecin, the reference substance, exhibited activity toward MCF-7, HeLa, HT-29 and KB cell lines, with IC_{50} range of 0.2-2.0 μg mL^{-1} (Table 1).

Results and Discussion

Mammea E/BB cyclo D (**1**) was isolated as a yellowish viscous oil, with a molecular formula $C_{24}H_{28}O_7$, established

Table 1. Cytotoxic activity of compounds **1-6**

Compound	IC_{50}/(μg mL^{-1})			
	MCF-7[a]	Hela[b]	HT-29[c]	KB[d]
1	Inactive	Inactive	Inactive	Inactive
2	Inactive	Inactive	Inactive	Inactive
3	1.33	2.56	0.78	1.33
4	4.64	3.52	4.06	4.06
5	Inactive	Inactive	Inactive	Inactive
6	Inactive	Inactive	Inactive	Inactive
Camptothecin	0.2-2.0	0.2-2.0	0.2-2.0	0.2-2.0

[a]MCF-7 (breast adenocarcinoma), [b]HeLa (human cervical cancer), [c]HT-29 (colon cancer) and [d] KB (human oral cancer).

by HREIMS analysis of its molecular ion $[M]^+$ at *m/z* 428.1813 (Calc. for $C_{24}H_{28}O_7$ *m/z* 428.1835). The UV spectrum of **1** showed absorption bands at 225, 280, 285, 300 and 373 nm suggesting the presence of conjugation in the molecule. The IR spectrum exhibited the characteristic of carbonyl (1738 and 1655 cm^{-1}) and hydroxyl (3454 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra revealed 24 carbons, including six methyls (δ 10.0, 10.6, 16.9, 21.1, 27.8 and 28.4), two methylenes (δ 28.6 and 29.6), five methines (δ 46.9, 73.0, 106.4, 115.8 and 126.8) and eleven non-hydrogenated carbons (δ 80.2, 100.9, 103.7, 106.5, 155.7, 156.7, 157.5, 159.3, 163.5, 170.3 and 210.8). The ^{1}H NMR spectral data showed a chelated hydroxyl proton at δ 14.44 assignable to 7-OH on the basis of HMBC correlations (Figure 2). The ^{1}H NMR spectrum also displayed a singlet signal at δ 6.30, which is a typical chemical shift for H-3 of 4-alkylcoumarin skeleton.[3,11] In addition, the ^{1}H NMR spectrum also showed the signals of chromene ring, 2-methyl-1-oxobutyl and 1-acetoxypropyl moieties. The ^{1}H NMR signals of chromene ring were appeared at δ 6.74 (1H, d, *J* 10.0 Hz, H-4″), 5.60 (1H, d, *J* 10.0 Hz, H-5″), 1.58 (3H, s, H-7″) and 1.56 (3H, s, H-8″), while the 2-methyl-1-oxobutyl group showed signals at δ 4.02 (1H, sextet, *J* 6.3 Hz, H-2‴), 1.80 (1H, m, H-3‴a), 1.45 (1H, m, H-3‴b), 1.26 (3H, d, *J* 6.3 Hz, H-5‴) and 1.06 (3H, t, *J* 7.2 Hz, H-4‴). Finally, the 1-acetonylpropyl moiety showed the ^{1}H NMR signals at δ 6.60 (1H, dd, *J* 6.8, 2.8 Hz, H-1′), 2.17 (3H, s, H-1′-COCH_3), 1.97 (1H, m, H-2′a), 1.78 (1H, m, H-2′b), and 1.07 (3H, t, *J* 7.2 Hz, H-3′). The locations of the three moieties were established based on the observed key HMBC correlations (Figure 2). The 1-acetoxypropyl unit was placed at C-4 due to the oxymethine proton H-1′ (δ 6.60) showed ^2J and ^3J correlation with C-4a (δ 100.9), C-4 (δ 157.5) and C-3 (δ 106.4) in the HMBC spectrum. In addition, the olefinic proton H-3 (δ 6.30) also showed ^2J and ^3J correlations with C-1′ (δ 73.0), C-2 (δ 159.3) and C-4a (δ 100.9). The chromene ring was located at C-5/C-6 because the olefinic proton H-4″ (δ 6.74) displayed HMBC correlations to C-5 (δ 155.7), C-6 (δ 106.5) and C-7 (δ 163.5). Finally, the hydroxyl group was located at C-4 because the chelated hydroxyl proton showed HMBC correlations to C-6 (δ 106.5), C-7 (δ 163.5) and C-8 (δ 103.7) and the 2-methyl-1-oxobutyl moiety had to be placed at C-8 by process of elimination. Therefore, the structure of mammea E/BB cyclo D was characterized as **1**.

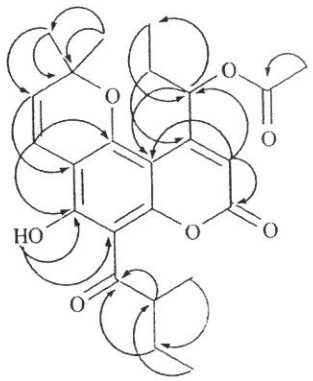

Figure 2. Selective HMBC correlations of compound 1.

The reported compounds were tested for their cytotoxicity against MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB human oral cancer) cell lines. The results are summarized in Table 1. Only two coumarins, **3** and **4**, were found to be active in this study. Suragin C (**3**) showed cytotoxic activities against all four cancer cell lines better than therapin B (**4**) (Table 1). It should be noted that the structural difference between suragin C (**3**) and therapin B (**4**) is only at C-6 (**3** possesses a geranyl group while **4** contains a prenyl group). The presence of a geranyl moiety seems to be important for enhancing the cytotoxic activity. The anticancer drug used as a standard in our cytotoxic assay is camptothecin, which has an IC_{50} in the range of 0.2-2.0 μg mL^{-1}.

It is worth noting that the genus *Mammea* of the family Guttiferae has been known to be rich in coumarins and xanthones,[1-5,9,12-17] with more than 30 compounds having been isolated from this genus. In this study, we have observed an additional new coumarin from the seeds of *M. siamensis*.

Acknowledgments

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