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PHYTOCHEMICAL STUDY OF *MAERUA SIAMENSIS* ROOTS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Department of

Pharmacognosy and Pharmaceutical Botany

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2010

Thesis Title	PHYTOCHEMICAL STUDY OF *MAERUA SIAMENSIS* ROOTS
By	Mr. Nawarat Chadchen
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Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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นวรัตน์ จัดเจน: การศึกษาทางพฤกษเคมีของรากแจง (PHYTOCHEMICAL STUDY OF *MAERUA SIAMENSIS* ROOTS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.รุทธ์ สุทธิศรี, 121 หน้า.

การศึกษาองค์ประกอบทางเคมีของรากแจก [*Maerua siamensis* (Kurz) Pax.] วงศ์ Capparidaceae สามารถแยกสารใหม่ในกลุ่มอินโดลแอลคาลอยด์ได้ 2 ชนิด คือ 7-hydroxy-6-methoxycyclobrassinone และ 7-hydroxycyclobrassinone กับสารที่เคยมีรายงานแล้วอีก 3 ชนิดคือ β-sitosterol, vanillin และ lupeol พิสูจน์โครงสร้างทางเคมีของสารที่สกัดได้เหล่านี้โดยอาศัยการวิเคราะห์เชิงสเปกตรัมด้วย UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่เคยมีรายงานมาก่อนแล้ว สาร 7-hydroxy-6-methoxycyclobrassinone มีความเป็นพิษระดับสูง (ค่า IC_{50} = 1.51 ไมโครกรัมต่อมิลลิลิตร) ในขณะที่สาร 7-hydroxycyclobrassinone มีความเป็นพิษในระดับปานกลาง (ค่า IC_{50} = 8.31 ไมโครกรัมต่อมิลลิลิตร) ต่อเซลล์มะเร็งปอดของมนุษย์ชนิด NCI-H187 นอกจากนี้ สาร 7-hydroxy-6-methoxycyclobrassinone ยังมีฤทธิ์ต้านเชื้อวัณโรค *Mycobacterium tuberculosis* โดยมีค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งเชื้อได้คือ 25 ไมโครกรัมต่อมิลลิลิตรอีกด้วย

ภาควิชา <u>เภสัชเวทและเภสัชพฤกษศาสตร์</u> ลายมือชื่อนิสิต........นวรัตน์.......จัดเจน.........

สาขาวิชา <u>เภสัชพฤกษศาสตร์</u> ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.......รุทธ์ สุทธิศรี........

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NAWARAT CHADCHEN: PHYTOCHEMICAL STUDY OF *MAERUA SIAMENSIS* ROOTS. THESIS ADVISOR: ASSOC.PROF. RUTT SUTTISRI, Ph.D., 121 PP.

Chemical investigation of the roots of *Maerua siamensis* (Kurz) Pax. (family Capparidaceae) led to the isolation of two new indole alkaloids named 7-hydroxy-6-methoxycyclobrassinone and 7-hydroxycyclobrassinone, together with three known compounds i.e. β-sitosterol, vanillin and lupeol. The structures of these isolated compounds were determined by spectroscopic analyses, including UV, IR, MS and NMR, and comparison with previously reported data. 7-Hydroxy-6-methoxycyclobrassinone was strongly active (IC_{50} = 1.51 µg/ml), while 7-hydroxycyclobrassinone was moderately active (IC_{50} = 8.31 µg/ml), against human small-cell lung cancer cell line (NCI-H187). In addition, 7-hydroxy-6-methoxycyclobrassinone also exhibited anti-tuberculosis activity against *Mycobacterium tuberculosis* with a minimum inhibitory concentration of 25 µg/ml.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ศูนย์วิทยทรัพยากร
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LIST OF SCHEMES

LIST OF ABBREVIATIONS

α	=	Alpha
β	=	Beta
br s	=	Broad singlet (for NMR spectra)
$^{\circ}C$	=	Degree Celsius
Calcd	=	Calculated
CC	=	Column chromatography
$CDCl_3$	=	Deuterated chloroform
CH_2Cl_2	=	Dichloromethane
cm	=	Centimeter
cm^{-1}	=	reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
2D NMR	=	Two dimensional Nuclear Magnetic Resonance
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
ddd	=	doublet of doublets of doublets (for NMR spectra)
DMSO-d_6	=	Deuterated dimethyl sulfoxide
δ	=	Chemical shift
ε	=	Molar absorptivity
ESI-MS	=	Electrospray Ionization Mass Spectrometry
EtOAc	=	Ethyl acetate
g	=	Gram
h	=	Hour
^1H NMR	=	Proton Nuclear Magnetic Resonance
^1H-^1H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
HMBC	=	Heteronuclear Multiple Bond Correlation
HR	=	High Resolution
HSQC	=	Heteronuclear Single Quantum Coherence
Hz	=	Hertz
IC_{50}	=	Median Inhibitory Concentration

IR	=	Infrared Spectrum
J	=	Coupling constant
KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
μg	=	Microgram
$\mu g/ml$	=	Microgram per milliliter
μl	=	Microliter
$[M]^+$	=	Molecular ion
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
MHz	=	Megahertz
MIC	=	Minimum inhibitory concentration
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
mp	=	melting point
MS	=	Mass Spectrometry
MW	=	Molecular weight
m/z	=	Mass to charge ratio
Na	=	Sodium
ν_{max}	=	Wave number at maximal absorption
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Enhancement Spectroscopy
ppm	=	Part-per-million
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
td	=	Triplet of doublets (for NMR spectra)

TLC = Thin Layer Chromatography

UV = Ultraviolet

CHAPTER I

INTRODUCTION

Members of the plant family Capparidaceae (Capparaceae) can be found in both the temperate and tropical regions of the world. In Thailand and certain other countries, some of these plants have been consumed as food. In Senegal the fruits of *Maerua pseudopetalosa*, which provide an excellent source of nutrients, are eaten by the native populations during the period of food shortage (Ayessou *et al*., 2009).

Several plants of this family are used medicinally and their extracts have been shown to be biologically active (Rajesh *et al*., 2009). For example, the ethanolic extract of the bark of *Crataeva religiosa,* a plant used in the Indian traditional medicine, have been shown to exhibit significant antifungal activity comparable to standard antifungal agents (Sahoo *et al*., 2008). Ethanolic extracts of the root bark of *Capparis spinosa* (Aghel, Rashidi and Mombeini, 2007) and the fruits of *C. moonii* (Ali *et al*., 2004) were hepatoprotective against carbon tetrachloride-induced liver damage in animal models. The immunostimulant activity of the ethanolic and water extracts of *C. zeylanica* leaves was explored and both extracts were able to prevent myelosuppression in mice treated with cyclophosphamide (Ghule *et al*., 2006). Similar extracts of the dried stem bark of *Crataeva nurvala* were effective in preventing pregnancy in rats (Bhaskar, *et al*., 2009). The bark and shoot of *Capparis decidua*, a xerophytic shrub which contains several alkaloids, are used as analgesic, anti-inflammatory, hypolipidemic and antidiabetic agents. The ethaholic extract of its aerial parts exhibited CNS depressant and anticonvulsant effects in animals (Goyal, Nagori and Sasmal, 2009). The roots of *Capparis sikkimensis* subsp. *formosana* yielded cappamensin A, a *2H*-1,4-benzoxazine-3(*4H*)-one, which displayed significant *in vitro* anticancer activity against several types of human tumor cell lines (Wu *et al*., 2003), whereas the roots of *Maerua subcordata* have been shown to possess contracting activity on the isolated guinea pig ileum (Samuelsson, Kyerematen and Farah, 1985).

Maerua siamensis (Kurz) Pax. (Thai name: Chaeng or Kaeng) is a plant belonging to the Capparidaceae and is the only member of its genus found in Thailand. The plant can be found growing in mixed deciduous forest, dry evergreen forest, dry

dipterocarp forest, open scrub jungle or on limestone hill at the altitude of not more than 400 meters. The roots of *M. siamensis* have been used in Thai folk medicine as analgesic, diuretic, analeptic and as a treatment for blurred vision, dizziness, malaria and wasting disease. Its stem bark has similar usages as the roots, and has also been used as antibacterial and to cure jaundice. Furthermore, the leaves and heartwood of this plant have been used to treat fever (ก่องกานดา ชยามฤต, 2528). However, no previous study has been performed on this plant species. Therefore, this investigation deals with the purification and identification of chemical compounds present in the roots of *M. siamensis*. The phytochemical data obtained in this study would contribute to the knowledge of chemical constituents of this plant family and would be valuable information in the fields of chemotaxonomy and phytochemistry.

The purposes of this research were as follows:

1. Isolation and purification of compounds from the roots of *Maerua siamensis*.

2. Determination of chemical structures and physical properties of each isolated compound.

CHAPTER II

HISTORICAL

1. The Family Capparidaceae

Plant species of the family Capparidaceae (or Capparaceae) can be found in warmer parts of the world, mainly in the tropics and subtropics, and in the Mediterranean. Several genera of this family are found in Africa where they are conspicuous as a major element in the flora of its dry regions. Capparidaceae is a medium-sized family of the order Capparales and is closely related to the family Brassicaceae (Cruciferae). The family constitutes approximately 17 genera and 470 species. The habit of plants of this family can be herb, shrub, tree or liana. In Thailand, examples of plant genera belonging to this family that can be found are *Capparis*, *Cleome*, *Crateva* and *Maerua*. Recently, the genus *Cleome* has been separated into a new family, the Cleomaceae (Heywood *et al*., 2007).

Several plants of this family have been used in traditional medicine of different countries. For example, in Thai traditional medicine *Capparis micracantha* (Thai name: Ching chi) is used as a treatment for fever, dermatitis, bronchitis and cancer. *Crataeva religiosa* (Thai name: Kum nam) has been used as diuretic, as a treatment for chloasma, dysentery and constipation, and to stimulate appetite (ก่องกานดา ชยามฤต, 2528).

2. The Genus *Maerua*

The genus *Maerua* comprises about 90 species found in tropical Asia, India and Africa. Only one *Maerua* species is found in Thailand. Members of this plant genus are shrubs or trees, although some can be scramblers or climbers. The stem of these plants has glabrous or pubescent surface, without spines and usually without branches. The leaves are palmately compound with 3-5 leaflets. The flowers are solitary or in corymbose or racemose inflorescences. The flowers have no petal, but there are 4 sepals which are joined at the base. The stamens are few to many. The ovary is at the end of a long stalk (gynophore). It is cylindrical, with 1 locule and numerous ovules. The stigma is disc-shaped. Its glabrous fruits are ellipsoidal in shape, with 1-3 large seeds (Chayamarit, 1991).

Some members of this genus can be consumed as food, for example, the leaves of *M. crassifolia*, which contain proteins, calcium, linoleic and α-linolenic acids, were recommended to be used as food and can contribute significantly to the nutrition of populations inhabiting the transition zone between the Sahara desert in North Africa and the Sudanian savannas in the south (Cook *et al*., 1998). On the other hand, various *Maerua* plants have been reported to be used in the treatment of intestinal diseases, mental illness, diarrhea, epilepsy and vomiting (Chhabra and Uiso, 1990). Two *Maerua* species native to the Middle East, *M. crassifolia* and *M. oblongifolia*, are used as traditional medicine in that region: the decoction of *M. crassifolia* leaves is used in toothache and intestinal diseases, whereas *M. oblongifolia* is used in the treatment of hypercholesterolemia (Rahman *et al*., 2004). Furthermore, the ethanolic extract of the whole plant of *M. crassifolia* displayed neuromuscular blocking and antitumor activities (Ibraheim, Ahmed and Ramadan, 2008).

3. *Maerua siamensis* (Kurz) Pax

The plant is a shrub or small tree up to 5-10 m high. Its branches are glabrous, whereas its palmately compound leaves are 3- or 5-folioate. The leaflets are subsessile. Their shape is obovate, oblong or linear, (2-)5-7(-12) cm by 1-3 cm. The leaf texture is either subcoriaceous or papery. They are glabrous on both sides. The leaf base is cuneate or obtuse, while the apex is emarginated or rounded with shortly mucronate tip. The leaf veins are very thin and finely reticulate. The slender petioles are 1.5-6.5 cm long. The flowers are in terminal or lateral corymb or raceme, or the inflorescence can be a short terminal panicle, or some flowers are solitary and appear in the axils of the upper leaves. The pedicels are 1.5-5.5 cm long. The bracts are small and linear. The ovate sepals are 7-10 long and 2-3 mm wide, with acuminate apex. They are glabrous on both sides, and are woolly at the margin. The stamens are 9-12, with robust filaments 10-15 mm long. The oblong anthers are 1.5-2 mm long. The gynophore is glabrous, 1.5-2 cm long. The cylindrical, glabrous ovary is 1.5-2 mm by 1 mm. The fruits are ellipsoidal or rounded, 2-2.5 cm by 1.3-1.5 cm. Its slender stipe is 4.5-7.5 cm long. The seeds are reniform in shape (Chayamarit, 1991).

A

B

C

D

Figure 1. *Maerua siamensis*

A) Tree, B) Fruits, C) Leaves and flowers, D) Roots

4. Indole Compounds in the Families Brassicaceae and Capparidaceae

Most indole compounds found in plants of the family Brassicaceae are secondary metabolites, called phytoalexins or phytoanticipins, which are involved in the defense against pathogens and pests. Both are antimicrobial substances that help to defend the plant by inhibiting the growth of invading microbes. Phytoalexins are produced *de novo* in the plant in response to abiotic stresses or biotic stresses such as infection by fungi or bacteria, whereas phytoanticipins are pre-formed inhibitors of infection, although the distinction between both types of compounds may not be obvious (Dixon, 2001). All currently known phytoalexins of this plant family contain an indole or oxindole nucleus, while phytoanticipins can be represented by a broader range of chemical structures (Pedras, Zheng and Strelkov, 2008).

Examples of the indole phytoalexins produced by plants in the family Brassicaceae are brassilexin (**8**), brassinin (**9**), camalexin (**12**), 1-methoxyspirobrassinin (**39**), 1-methoxyspirobrassinol (**40**), 1-methoxyspirobrassinol methyl ether (**41**) and spirobrassinin (**49**). These compounds, found in several species of *Brassica* (Gross *et al*., 1994; Pedras *et al*., 2008) and *Raphanus* (Monde, Takasuki and Shirata, 1995), have been demonstrated to possess significant antiproliferative activity against various cancer cells (Mezencev *et al*., 2003). Biswasalexins A1 (**3**) and A2 (**4**) are phytoalexins produced from the sodium chloride and UV stressed salt cress plants (*Thellungiella halophila*) that have been shown to exhibit antifungal activity (Pedras *et al*., 2009). Cyclobrassinin (**16**), biologically derived from the oxidative cyclization of brassinin, has been shown to significantly inhibit the formation of preneoplastic mammary lesions and brassinin may be effective as a chemopreventive agent during the initiation and promotion phases of carcinogenesis (Mehta *et al*., 1995). Cyclobrassinin, together with 44 other metabolites, can also be identified in the roots of canola (*Brassica napus*) infected with the soilborne phytopathogen *Plasmodiophora brassicae* (Pedras *et al*., 2008).

Capparidaceae is a medium-sized family closely related to the family Brassicaceae. Although it is not as economically significant as the crucifers, several members of the Capparidaceae are consumed as food plants. Indole compounds have also been identified as constituents of this plant family. Capparilosides A (**56**) and B

(**57**) are two glucose-containing 1H-indole-3-acetonitrile compounds found in the fruits of *Capparis spinosa* (Calis, Kuruuzum and Ruedi, 1999). Two more indole glycosides (**60** and **61**) were isolated from the roots of another *Capparis* species, *C. tenera* (Su *et al.*, 2007). And, recently, isolation of the whole plant of *C. himalayensis*, a Chinese plant of which its root barks, leaves and fruits are used in traditional medicine for the treatment of rheumatism, yielded two alkaloids, capparin A (**58**) and B (**59**) (Li *et al.*, 2008). Capparin A possesses the feature of spirobrassinin (**49**), previously reported from a plant in the family Brassicaceae.

The distribution of indole compounds in the families Brassicaceae and Capparidaceae is shown in **Tables 1** and **2**, and their chemical structures are shown in **Figures 2** and **3**.

Table 1. Distribution of indole compounds in the family Brassicaceae

Compound	Source	Plant part	References
Arvelexin (**1**)	Thlaspi arvense	Leaves	Pedras, Chulama and Suchy, 2003
Biswasalexin A1 (**2**)	*Thellungiella halophila*	Aerial parts	Pedras *et al*., 2009
Biswasalexin A2 (**3**)			
Brassicanal A (**4**)	*Brassica napus* var. *rapifera*	Tuber	Pedras *et al*., 2004
Brassicanal B (**5**)	*B. campestris*		Monde *et al*., 1990a
Brassicanal C (**6**)	*B. oleracea*	n.i.	Monde, Sasaki and Takasugi, 1991b
Brassicanate A (**7**)	*B. napus* var. *rapifera*	Tuber	Pedras *et al*., 2004
Brassilexin (**8**)	*B. juncea*	Leaves	Devys *et al*., 1998
Brassinin (**9**)	*B. campestris* var. *pekinensis*	n.i.	Takasugi, Katsui and Shirata, 1986
Brassitin (**10**)	*Raphanus sativus* var. *hortensis*	Roots	Monde *et al*., 1995
Brussalexin A (**11**)	*Brassica oleracea*	n.i.	Pedras, Zheng and Sarwar, 2007
Camalexin (**12**)	*Camelina sativa*	Leaves	Browne *et al*., 1991
Caulilexin A (**13**)	*Brassica oleracea*	n.i.	Pedras *et al*., 2006
Caulilexin B (**14**)			
Caulilexin C (**15**)			
Cyclobrassinin (**16**)	*B. campestris* var. *pekinensis*	n.i.	Takasugi, Katsui and Shirata, 1986

n.i. = not indicated

Table 1. (continued)

Compound	Source	Plant part	References
Cyclobrassinin sulfoxide (**17**)	*Brassica juncea*	Leaves	Devys *et al*., 1990b
Cyclobrassinone (**18**)	*B. oleracea* var *gongylodes*	Stems	Gross, Porzel and Schmidt, 1994
Dehydrocyclobrassinin (**19**)	*B. napus*	Roots	Pedras *et al*., 2008
Dioxibrassinin (**20**)	*B. oleracea*	n.i.	Monde *et al*., 1991b
Epiglucoisatisin (**21**)	*Isatis tinctoria*	Seeds	Frechard *et al*., 2001
Glucobrassicin (**22**)	*Brassica oleracea*	n.i.	Gmelin, Saarivirta and Virtanen, 1960
Glucoisatisin (**23**)	*Isatis tinctoria*	Seeds	Frechard *et al*., 2001
3'-Hydroxyepiglucoisatisin (**24**)			
4-Hydroxyglucobrassicin (**25**)	*Brassica oleracea*	n.i.	Truscott, Burke and Minchinton, 1982
3'-Hydroxyglucoisatisin (**26**)	*Isatis tinctoria*	Seeds	Frechard *et al*., 2001
Indolyl-3-acetonitrile (**27**)	*Brassica rapa*	Aerial parts/Whole plant	Wakabayashi *et al*., 1985
Isalexin (**28**)	*B. napus* var. *rapifera*	Tuber	Pedras *et al*., 2004
1-Methoxybrassenin A (**29**)	*B. oleracea* var. *capitata*	n.i.	Monde *et al*., 1991a
1-Methoxybrassenin B (**30**)			
1-Methoxybrassinin (**31**)	*B. campestris* var. *pekinensis*	Aerial parts	Takasugi, Katsui and Shirata, 1986
4-Methoxybrassinin (**32**)	*B. oleracae*	n.i.	Monde *et al*., 1990b

n.i. = not indicated

Table 1. (continued)

Compound	Source	Plant part	References
1-Methoxybrassitin (33)	*B. campestris* var. pekinensis	Aerial parts	Takasugi *et al*., 1988
1-Methoxycamalexin (34)	*Camelina sativa*	Leaves	Browne *et al*., 1991
6-Methoxycamalexin (35)	*Capsella bursa-pastoris*	Leaves	Jimenez, Ayer and Tewari, 1997
4-Methoxycyclobrassinin (36)	*Brassica napus*	Roots	Pedras *et al*., 2008
4-Methoxydehydrocyclobrassinin (37)	*B. campestris*	n.i.	Monde *et al*., 1994
4-Methoxyglucobrassicin (38)	*B. oleracea*	n.i.	Truscott *et al*., 1982
1-Methoxyspirobrassinin (39)	*B. oleracea*	Stems	Gross *et al*., 1994
1-Methoxyspirobrassinol (40) 1-Methoxyspirobrassinol methyl ether (41)	*Raphanus sativus* var. *hortensis*	Roots	Monde *et al*., 1995
1-Methylcamalexin (42)	*Capsella bursa-pastoris*	Leaves	Jimenez *et al*., 1997
Methyl indole-3-carboxylate (43)	*Brassica napus*	Roots	Pedras *et al*., 2008
Methyl 1-methoxyindole-3-Carboxylate (44)	*Wasabia japonica*	n.i.	Somei *et al*., 2001
Neoglucobrassicin (45)	*Brassica napus*	Root barks	Gmelin and virtanen, 1962
Rapalexin A (46) Rapalexin B (47)	*B. rapa*	Leaves	Pedras, Zheng and Gadagi, 2007
Rutalexin (48)	*Brassica napus* var. *rapifera*	Tuber	Pedras *et al*., 2004
Spirobrassinin (49)	*Rhaphanus sativus* var. *hortensis*	Roots	Takasugi et al., 1987

n.i. = not indicated

Table 1. (continued)

Compound	Source	Plant part	References
Sinalexin (**50**)	*Sinapis alba*	Leaves	Pedras and Smith, 1997
Sinalbin A (**51**)			Pedras and Zaharia, 2000
Sinalbin B (**52**)	*S. alba*	Leaves	Pedras and Zaharia, 2000
Wasalexin A (**53**)	*Wasabia japonica*	n.i.	Pedras *et al*., 1999
Wasalexin B (**54**)			

n.i. = not indicated
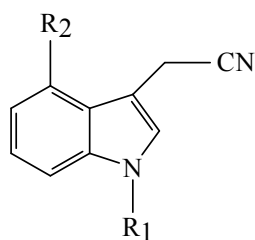
Arvelexin (1): $R_1 = H$, $R_2 = H$

Caulilexin C (15): $R_1 = OCH_3$, $R_2 = H$

Indolyl-3-acetonitrile (27): $R_1 = H$, $R_2 = OCH_3$
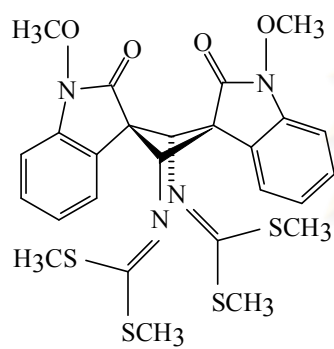
Biswasalexin A1 (2)
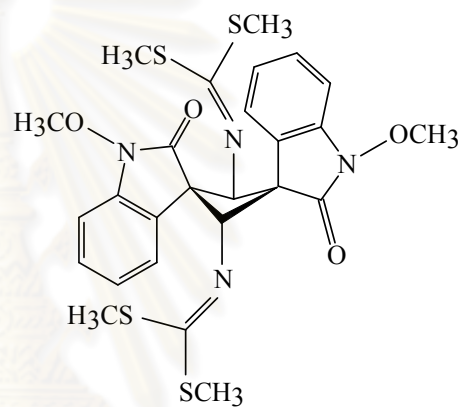
Biswasalexin A2 (3)
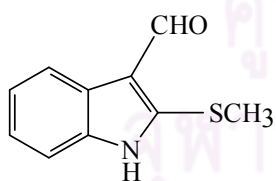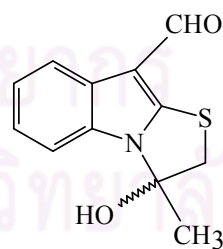
Brassicanal A (4)

Brassicanal B (5)

Figure 2. Chemical structures of indole compounds in the family Brassicaceae
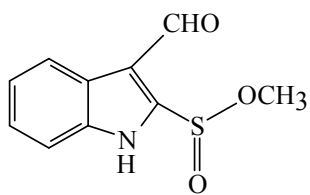
Brassicanal C (**6**)
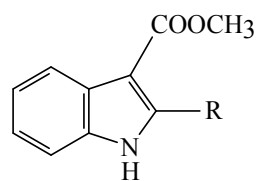
Brassicanate A (**7**): R = H

Methyl indole-3-carboxylate (**43**): R = SCH$_3$
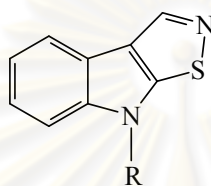
Brassilexin (**8**): R = H

Sinalexin (**50**): R = OCH$_3$
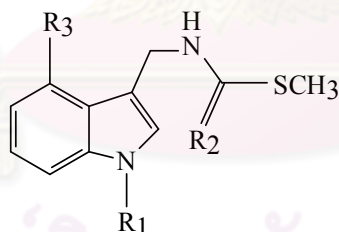
Brassinin **9**): R$_1$ = H, R$_2$ = S, R$_3$ = H

Brassitin (**10**): R$_1$ = H, R$_2$ = O, R$_3$ = H

1-Methoxybrassinin (**31**): R$_1$ = OCH$_3$, R$_2$ = S, R$_3$ = H

4-Methoxybrassinin (**32**): R$_1$ = H, R$_2$ = S, R$_3$ = OCH$_3$

1-Methoxybrassitin (**33**): R$_1$ = OCH$_3$, R$_2$ = O, R$_3$ = H

Figure 2. (continued)
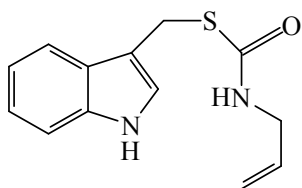
Brussalexin A (11)
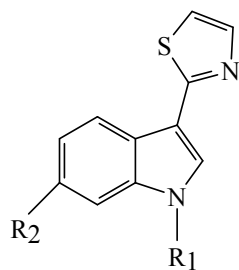
Camalexin (12): R₁ = H, R₂ = H

1-Methoxycamalexin (34): R₁ = OCH₃, R₂ = H

6-Methoxycamalexin (35): R₁ = H, R₂ = OCH₃

1-Methylcamalexin (42): R₁ = CH₃, R₂ = H
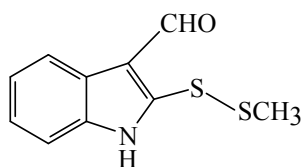
Caulilexin A (13)
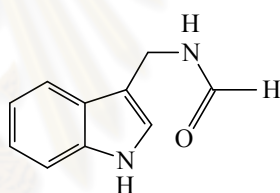
Caulilexin B (14)
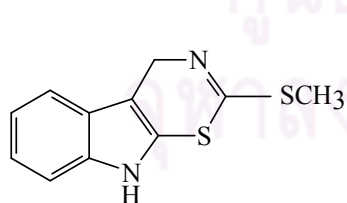
Cyclobrassinin (16)
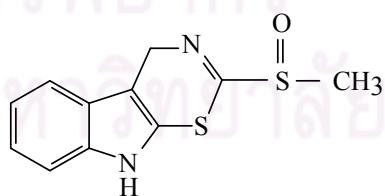
Cyclobrassinin sulfoxide (17)

Figure 2. (continued)
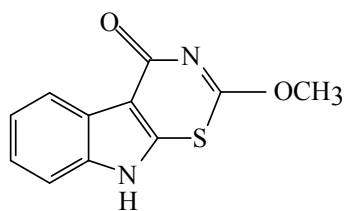
Cyclobrassinone (**18**)
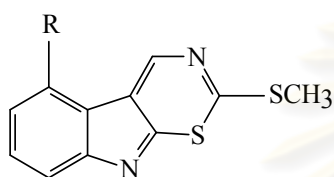
Dehydrocyclobrassinin (**19**): R = H

4-Methoxydehydrocyclobrassinin (**37**): R = OCH₃
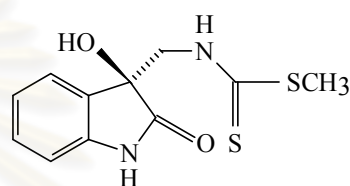
Dioxibrassinin (**20**)
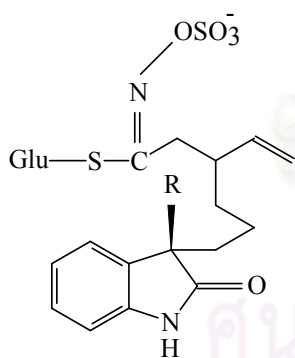
Epiglucoisatisin(S) (**21**): R = H

3'-Hydroxyepiglucoisatisin (**24**): R = OH
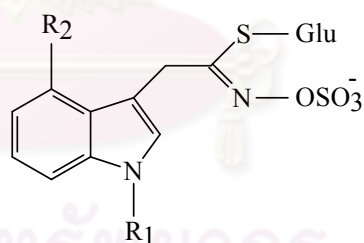
Glucobrassicin (**22**): R₁ = H, R₂ = H

4-Hydroxyglucobrassicin (**25**): R₁ = H, R₂ = OH

4-Methoxyglucobrassicin (**38**): R₁ = H, R₂ = OCH₃

Neoglucobrassicin (**45**): R₁ = OCH₃, R₂ = H

Figure 2. (continued)
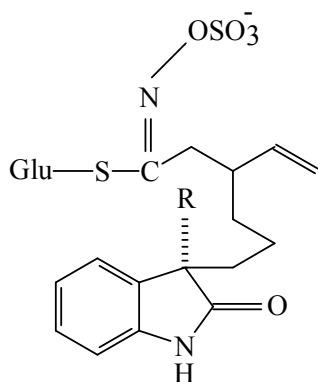
Glucoisatisin (**23**): R = H

3'-Hydroxyglucoisatisin (**26**): R = OH
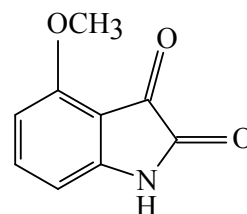
Isalexin (**28**)
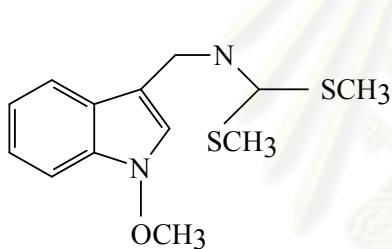
1-Methoxybrassenin A (**29**): R = H$_2$ 1-Methoxybrassenin B (**30**): R = O
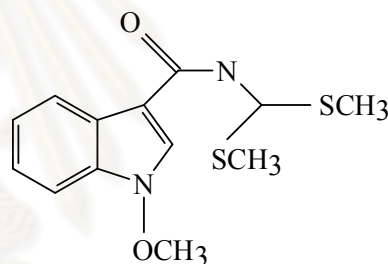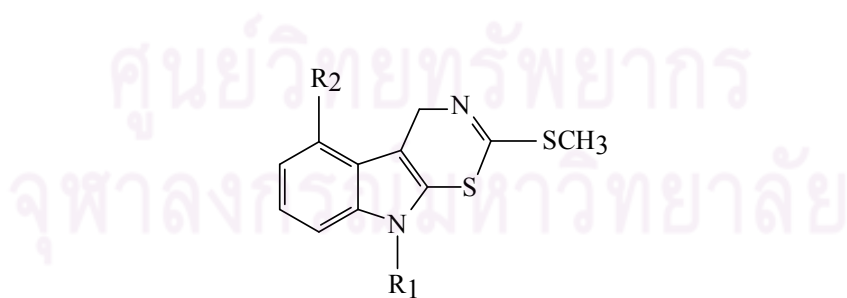
4-Methoxycyclobrassinin (**36**): R$_1$ = H, R$_2$ = OCH$_3$

Sinalbin B (**52**) R$_1$ = OCH$_3$, R$_2$ = H

Figure 2. (continued)
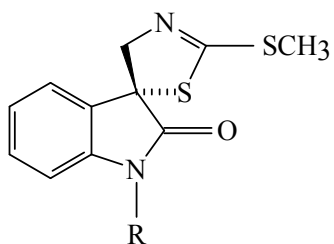
1-Methoxyspirobrassinin (**39**): R = OCH₃

Spirobrassinin (**49**) : R = H

1-Methoxyspirobrassinol (**40**)
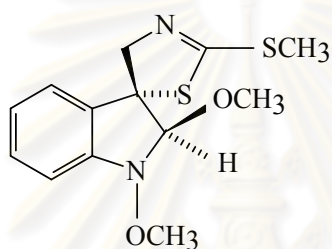
1-Methoxyspirobrassinol methyl ether (**41**)
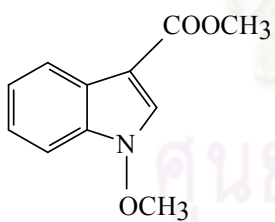
Methyl 1-methoxyindole-3-carboxylate (**44**)
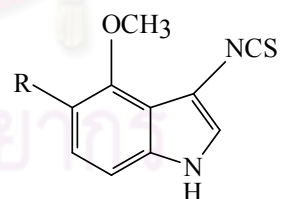
Rapalexin A (**46**): R = H

Rapalexin B (**47**): R = OH

Figure 2. (continued)
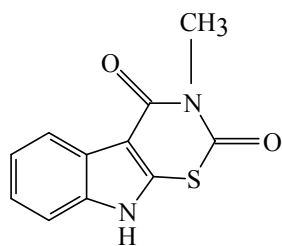
Rutalexin (48)
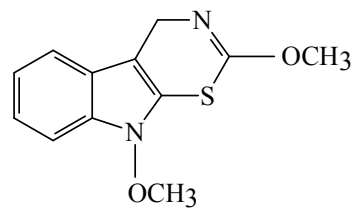
Sinalbin A (51)
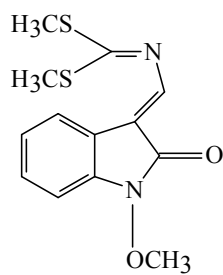
Wasalexin A (53)

Wasalexin B (54)

Figure 2. (continued)
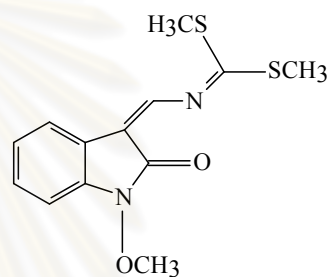
Table 2. Distribution of indole compounds in the family Capparideceae

Compound	Source	Plant part	References
Cappariloside A (56)	*Capparis spinosa*	Fruits	Calis *et al.*, 1999
Cappariloside B (57)			
Capparin A (58)	*C. himalayensis*	Whole plant	Li *et al.*, 2008
Capparin B (59)			
4-(β-D-Glucopyranoside)-1H-indole-3-carboxaldehyde (60)	*C. tenera*	Roots	Su *et al.*, 2007
4-(β-D-Glucopyranoside)-1H-indole-3-acetamide (61)			
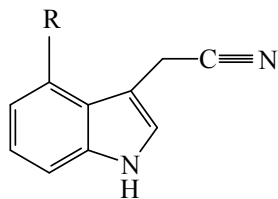
Cappariloside A (**55**): R = O-glu

Cappariloside B (**56**): R = O-glu-glu
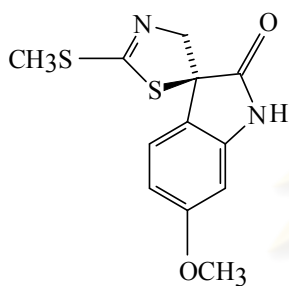
Capparin A (**57**)
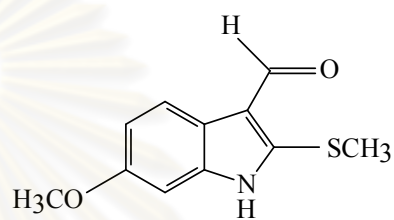
Capparin B (**58**)
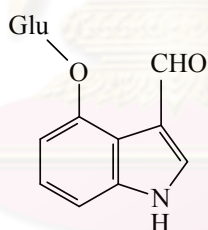
4-(β-D-Glucopyranoside)-1H-indole-3-carboxaldehyde (**60**)
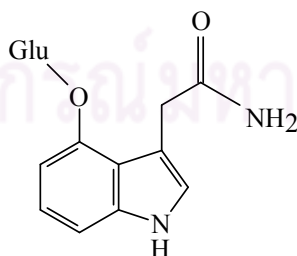
4-(β-D-Glucopyranoside)-1H-indole-3-acetamide (**61**)

Figure 3. Chemical structures of indole compounds in the family Capparidaceae

5. Chemical Constituents of Plants in the Genus *Maerua*

Currently, there has been a study on the quaternary ammonium compounds in a number of *Maerua* species (McLean, Blunden and Jewers, 1996) and chemical investigations on three *Maerua* plants, namely, *M. arenaria, M. crassifolia* and *M. oblongifolia*. These phytochemical studies have shown that the plants contain steroids and triterpenoids, fatty acids, long-chain hydrocarbons and glycolipids, flavonoids, alkaloids, ionol glucosides, phenolic and benzoic acid derivatives.

M. arenaria is a shrub found growing in India, Pakistan and Sri Lanka and is very closely related to *M. oblongifolia*, which grows in Arabia and tropical Africa. Phytochemical investigation of the plant yielded three phenolic compounds: 4-hydroxybenzoic acid (**78**), methyl grevillate (**92**) and 1-*O*-coumaroylglycerol (**68**), the steroids β -sitosterol (**98**) and its glucoside(**99**), the triterpenoid ursolic acid (**106**), a fatty acid, dodecanoic acid (**73**), and a diglyceride: glycerol 1,3-didodecanoate (**75**) (Ali *et al*., 2008).

M. crassifolia, a medicinal plant found in both Africa and South Asia, was investigated and found to contain flavonoids including kaempferol (**85**) and its glycosides (**86** and **87**) and quercetin (**95**) and its glycoside (**96**), the triterpenoids α-amyrin (**62**), lupeol acetate and (**89**) lupeol palmitate (**90**), the steroid β-sitosterol palmitate (**100**) a phenolic compound, guaiacylglycerol (**76**), glycolipids and long chain hydrocarbons. Furthermore, five ionol glucosides (**69**, **71**, **72**, **84** and **93**) were also reported (Bishay *et al*., 1990; Ibraheim and Abdallah, 1994; Ramadan *et al*., 1998; Ramadan *et al*., 1999; Ibraheim, 2002; Ibraheim *et al*., 2008).

M. oblongifolia is a shrub or scandent shrub commonly found growing in Saudi Arabia. Three triterpenoids were reported as its chemical constituents including betulinal (**63**), betulinol (**64**) and wallichenol (**107**) (Abdel-Mogib, 1999).

Investigation of the quaternary ammonium compounds in a number of African *Maerua* species indicated that several of these plants, namely, *M. subcordata*, *M. decumbens*, *M. edulis*, *M. pseudopetalosa*, previously classified as belonging to a different genus i.e. *Courbonia*, were shown to contain prolinebetaine ethyl ester (**96**) and tetramethylammonium. These two compounds were not detected in any of the other *Maerua* species examined (McLean *et al*., 1996).

Chemical constituents of these *Maerua* species are shown in **Table 4**, and their chemical structures are presented in **Figure 4**.

Table 3. Chemical constituents of plants in the genus *Maerua*

Compound	Source	Plant part	References
α-Amyrin (**62**)	*Maerua crassifolia*		Ibraheim *et al*., 2008
Betulinal (**63**)	*M. oblongifolia*	Aerial parts	Abdel-Mogib, 1999
Betulinol (**64**)			
Ceryl alcohol (**65**)	*M. crassifolia*		Ibraheim *et al*., 2008
Choline (**66**)	*M. kirkii*, *M. bussei*	Aerial parts/Whole plant	McLean *et al*., 1996
1-*O*-Coumaryl glycerol (**67**)	*M. arenaria*	n.i.	Ali *et al*., 2008
3-[(3'*R*,4'*R*,5'*S*,6'*S*)-1',3',4',5'-Tetrahydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**68**)	*M. crassifolia*	Aerial parts	Ramadan *et al*., 1998
1,2 3-Dimethoxy tricosa-6-one (**69**)			Ibraheim *et al*., 2008
-3-[(4'*R*,6'*S*)-1,4-Dihydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**70**)			Ramadan *et al*., 1998
3-[(4'*R*,5'*S*,6'*S*)-4',5'-Dihydroxy-2',2',6'-trimethylcyclohexyl)-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**71**)			Ibraheim and Abdallah, 1994

n.i. = not indicated

Table 3. (continued)

Compound	Source	Plant part	References
Dodecanoic acid (**72**)	*Maerua arenaria*	n.i.	Ali *et al.*, 2008
Glycerol 1,3-didodecanoate (**73**)	*Maerua arenaria*	n.i.	Ali *et al.*, 2008
Glycinebetaine (**74**)	*M. decandra, M. denhardtiorum, M. edulis, M. endlichlii, M. oblongifolia, M.pseudopetalosa , M. subcordata*	Aerial parts/Whole plant	McLean *et al.*, 1996
Guaiacylglycerol (**75**)	*M. crassifolia*	n.i.	Ramadan *et al.*, 1999
Hexacosanamide (**76**)		Aerial parts	Ibraheim, 2002
4-Hydroxybenzoic acid (**77**)	*M. arenaria*	n.i.	Ali *et al.*, 2008
3-[(4'*R*,6'*S*)-4'-Hydroxy-2',2',6'-trimethylcyclohexanyl]-1-methyl-(2*E*)-propen-1-ol (**78**)	*M. crassifolia*	Aerial parts	Ibraheim, 1995
3-Hydroxy-1,1-dimethyl-pyrrolidinium (**79**)	*M. acuminata, M. aethiopica, M. angolensis, M. calantha, M. crassifolia, M. decandra, M. decumbens, M. denhardtiorum,*	Aerial parts/Whole plant	McLean *et al.*, 1996

n.i. = not indicated

Table 3. (continued)

Compound	Source	Plant part	References
	Maerua edulis, *M. endlichlii,* *M. friesii,* *M. glauca,* *M. grantii,* *M. holstii* *M. juncea,* *M. kaessneri,* *M. ovalifolia,* *M. parvifolia,* *M. polyandra,* *M. prittwitzii,* *M. pseudopetalosa,* *M. sessiliflora,* *M. subcordata,* *M. triphylla* var. *calophylla,* *M. triphylla* var. *johannis,* *M. triphylla* var. *pubescens,* *M. triphylla* var. *triphylla,*	Aerial parts/Whole plant	McLean *et al.*, 1996

Table 3. (continued)

Compound	Source	Plant part	References
cis-3-Hydroxyprolinebetaine (**80**)	*Maerua acuminata,*		
trans-3-Hydroxyproline-betaine (**81**)	*M. aethiopica,* *M. angolensis,* *M. bussei,* *M. calantha,* *M. crassifolia,* *M. decandra,* *M. decumbens,* *M. denhardtiorum,* *M. edulis,* *M. eminii,* *M. endlichlii,* *M. friesii,* *M. glauca,* *M.* grantii, *M. holstii,* *M. juncea,* *M. kaessneri,* *M. kirkii,* *M. ovalifolia,* *M. parvifolia,* *M. polyandra,* *M. prittwitzii,* *M. pseudopetalosa,* *M. sessiliflora,*	Aerial parts/Whole plant	McLean *et al.*, 1996

Table 3. (continued)

Compound	Source	Plant part	References
	Maerua triphylla var. *calophylla,* *M. triphylla* var. *johannis,* *M. triphylla* var. *pubescens,* *M. triphylla* var. *triphylla*		McLean *et al.*, 1996
3-[(4'*R*,6'*S*)-4'-Hydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**82**)	*M. crassifolia*	Aerial parts	Ibraheim, 1995
Kaempferol (**83**)	*M. crassifolia*	Aerial parts	Bishay *et al.*, 1990
Kaempferol 3-*O*-rhammnosyl-galactoside (**84**)			Bishay *et al.*, 1990
Kaempferol 3-*O*-rhamnosyl-glucoside (**85**)			Ibraheim and Abdallah, 1994
Lyoniresinol 9'-β–D-glucopyranoside (**86**)			Bishay *et al.*, 1990
Lupeol acetate (**87**)			Ibraheim *et al.*, 2008
Lupeol palmitate (**88**)			Ramadan *et al.*, 1999
6-N-Methyl adenosine-9-β–D-glucoside (**89**)			
Methyl grevillate (**90**)	*M. arenaria*		Ali *et al.*, 2008

Table 3. (continued)

Compound	Source	Plant part	References
3-[(4'*R*,5'*S*,6'*S*)-1',4',5'- Trihydroxy-2',2',6'- trimethyl cyclohexyl]-1-methyl-(2*E*)- propen-1-yl β–D- glucopyranoside (**91**)	*Maerua crassifolia*	Aerial parts	Ramadan *et al*., 1998
Pentacosanamide (**92**)			Ibraheim, 2002
Quercetin (**93**)			Bishay *et al*., 1990
Quercetin 3-galacto- rhamnoside (**94**)			Ibraheim and Abdallah, 1994
Prolinebetaine (**95**)	*M. decandra,* *M. decumbens,* *M. denhardtiorum,* *M. edulis* *M. eminii,* *M. endlichlii,* *M. friesii,* *M. glauca,* *M. grantii,* *M. holstii,* *M. juncea,* *M. kaessneri,* *M. ovalifolia,* *M. parvifolia,* *M. Polyandra,* *M. Prittwitzii,* *M. pseudopetalosa*	Aerial parts/Whole plant	McLean *et al*., 1996

Table 3. (continued)

Compound	Source	Plant part	References
	Maerua sessiliflora, *M. subcordata*, *M. triphylla* var. *calophylla*, *M. triphylla* var. *johannis*, *M. triphylla* var. *pubescens*, *M. triphylla* var. *triphylla*	Aerial parts/Whole plant	McLean *et al*., 1996
Prolinebetaine ethyl ester (**96**)	*M. edulis*, *M. pseudopetalosa*, *M. subcordata*	Aerial parts/Whole plant	McLean *et al*., 1996
β-Sitosterol (**97**)	*M. arenaria*	n.i.	Ali *et al*., 2008
β-Sitosterol 3-O-β-D-glucopyranoside (**98**)			
β-Sitosterol palmitate (**99**)	*M. crassifolia*	Aerial parts	Ibraheim *et al*., 2008
Tetracosanamide (**100**)			Ibraheim, 2002
Triacontane (**101**)			Ibraheim *et al*., 2008
3,4,5-Trimethoxyphenol-1-*O*-β-D-glucopyranoside (**102**)		n.i.	Ramadan *et al*., 1999
Ursolic acid (**103**)	*M. arenaria*	n.i.	Ali *et al*., 2008
Wallichenol (**104**)	*M. oblongifolia*	Aerial parts	Abdel-Mogib, 1999

n.i. = not indicated
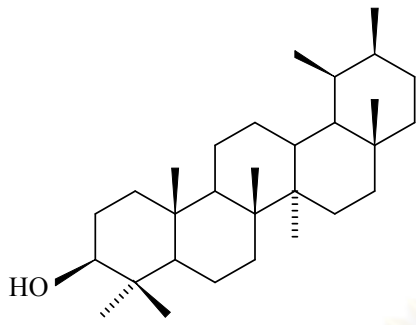
α-Amyrin (**62**)

Betulinal (**63**)

Betulinol (**64**)

H3C — (CH2)25 — CH3
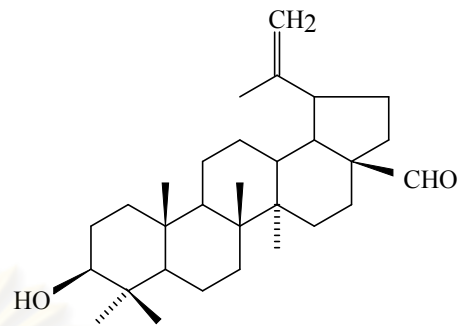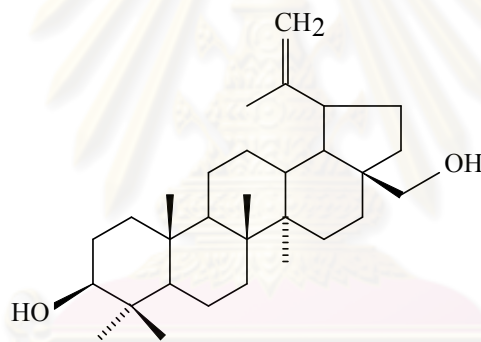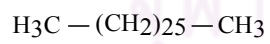
Ceryl alcohol (**65**)
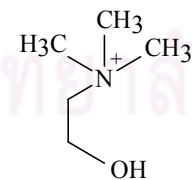
Choline (**66**)

Figure 4. Chemical constituents of *Maerua* species
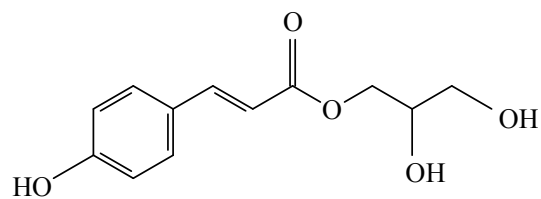
1-*O*-Coumaroyl glycerol (**67**)
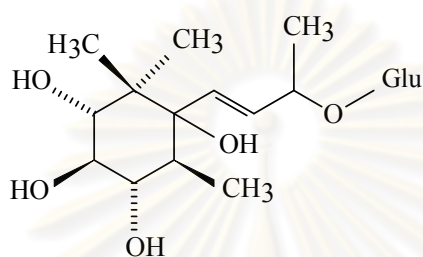
3-[(3'*R*,4'*R*,5'*S*,6'*S*)-1',3',4',5'-Tetrahydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**68**)
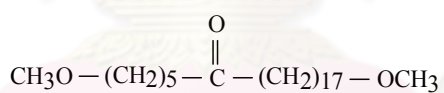
1,2,3-Dimethoxy tricosa-6-one (**69**)
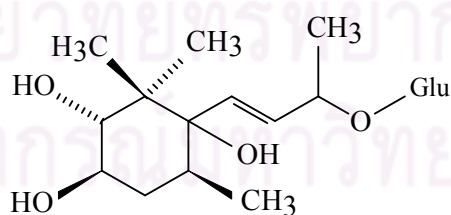
3-[(4'*R*,6'*S*)-1',4'-Dihydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**70**)

Figure 4. (continued)
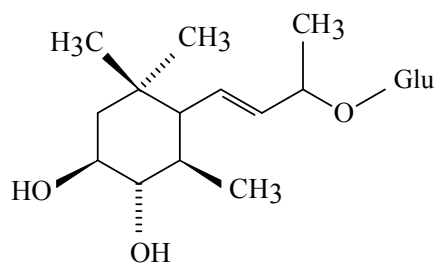
3-[(4'R,5'S,6'S)-4',5'-Dihydroxy-2',2',6'-trimethylcyclohexyl)-1-methyl-(2E)-propen-1-yl β–D-glucopyranoside (**71**)

$$HOOC - (CH_2)_{10} - CH_3$$

Dodecanoic acid (**72**)

$$H_3C - (CH_2)_{10} - \overset{\overset{\displaystyle O}{\|}}{C} - O - CH_2 - \overset{\overset{\displaystyle OH}{|}}{CH} - CH_2 - O - (CH_2)_{10} - CH_3$$
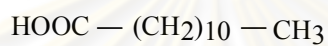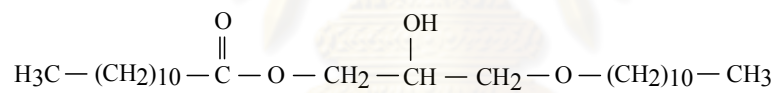
Glycerol 1,3-didodecanoate (**73**)
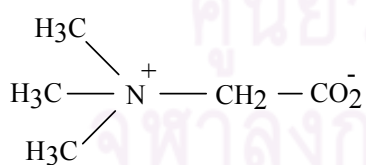
Glycinebetaline (**74**)
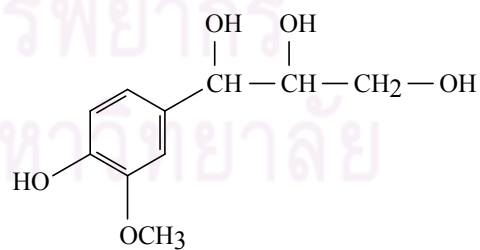
Guaiacylglycerol (**75**)

Figure 4. (continued
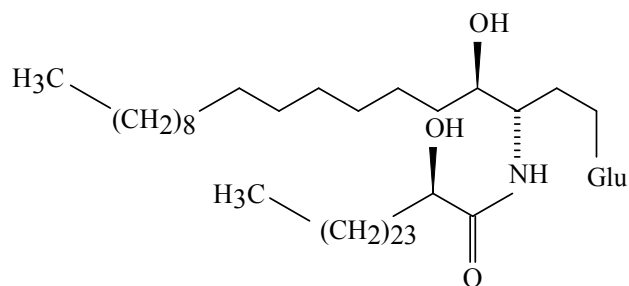
N-[(1*S*,2*R*,3*E*,7*E*)-1-[(β-D-Glucopyranosyloxy)methyl]-2-hydroxy-3,7-heptadecadien-1-yl]-2-hydroxy-(2*R*) (**76**)
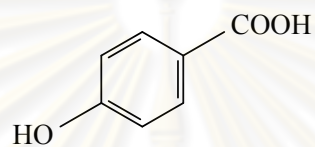
4-Hydroxybenzoic acid (**77**)
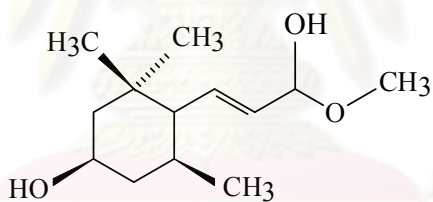
3-[(4'*R*,6'*S*)-4'-Hydroxy-2',2',6'-trimethylcyclohexanyl]-1-methyl-(2*E*)-propen-1-ol (**78**)
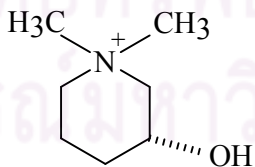
3-Hydroxy-1,1-dimethylpyrrolidinium (**79**): R$_1$ = H, R$_2$ = OH

Figure 4. (continued)
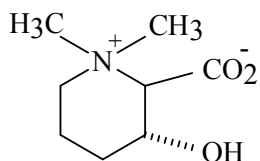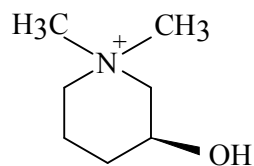
cis-3-Hydroxyprolinebetaine (**80**)   *trans*-3-Hydroxyprolinebetaine (**81**)
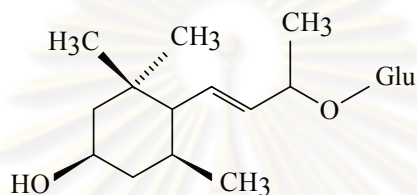
3-[(4'*R*,6'*S*)-4'-Hydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2*E*)-propen-1-yl β–D-glucopyranoside (**82**)
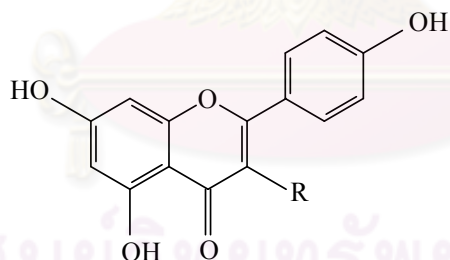
Kaempferol (**83**): R_1 = OH, R_2 = H

Kaempferol 3-*O*-rhamnosylgalactoside (**84**): R = O-Gal-Rha

Kaempferol 3-*O*-rhamnosylglucoside (**85**): R = O-Glu-Rha

Figure 4. (continued)
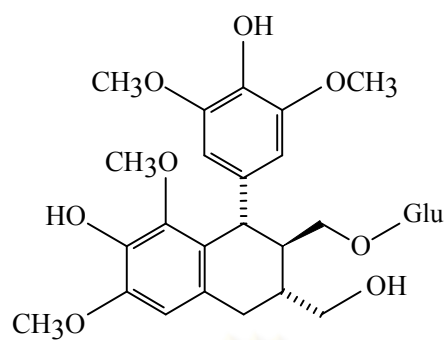
Lyoniresinol 9'-β-D-glucopyranoside (**86**)
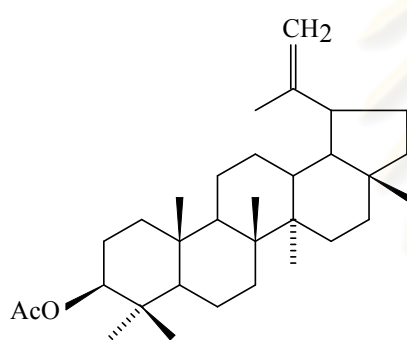
Lupeol acetate (**87**)
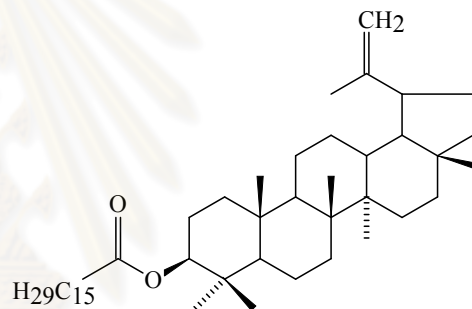
Lupeol palmitate (**88**)

6-N-Methyl adenosine-9-β-D-glucoside (**89**)
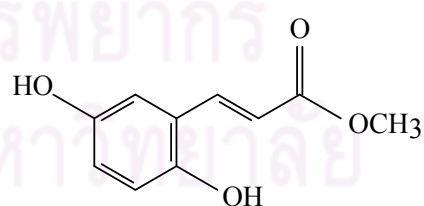
Methyl grevillate (**90**)

Figure 4. (continued)
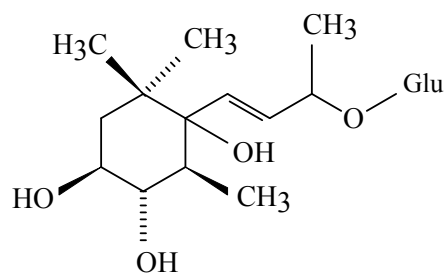
3-[(4'R,5'S,6'S)-1',4',5'-Trihydroxy-2',2',6'-trimethylcyclohexyl]-1-methyl-(2E)-propen-1-yl

β–D-glucopyranoside (**91**)
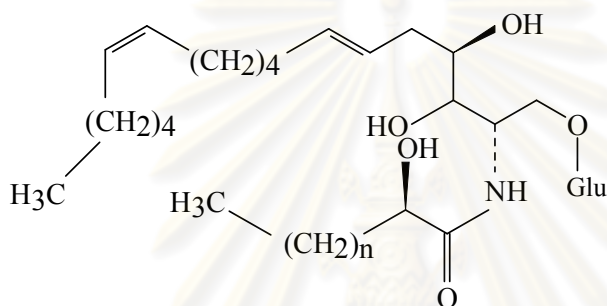
(2R)-N-[(1S,2S,3R,5E,11Z)-1-[(β-D-Glucopyranosyloxy)methyl]-2,3-dihydroxy-5,11-

heptadecadien-1-yl]-2-hydroxy (**92**): n = 22

(2R)-N-[(1S,2S,3R,5E,11Z)-1-[(β-D-Glucopyranosyloxy)methyl]-2,3-dihydroxy-5,11-

heptadecadien-1-yl]-2-hydroxy (**100**): n = 21
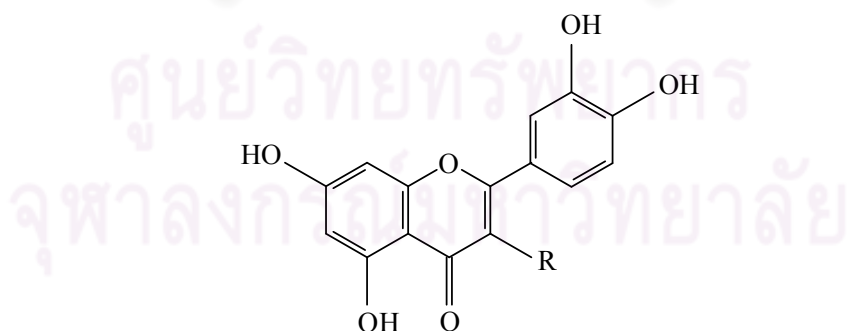
Quercetin (**93**): R = OH

Quercetin-3-O-galactorhamnoside (**94**): R = O- Rha-Gal

Figure 4. (continued)
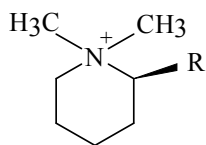
Prolinebetaine (**95**): R = COO⁻

Prolinebetaine ethyl ester (**96**): R = COOCH₂CH₃
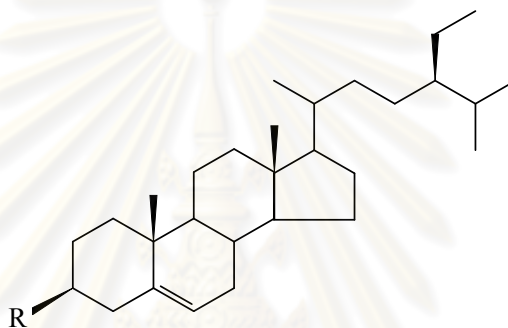
β-Sitosterol (**97**): R = OH

β-Sitosterol 3-*O*-β-D-glucopyranoside (**98**): R = O-glu
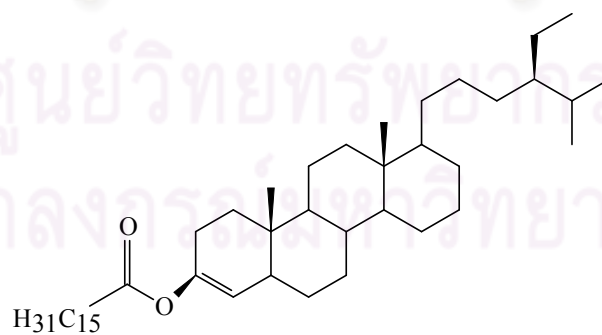
β-Sitosterol palmitate (**99**)

Figure 4. (continued)

H₃C ─ (CH₂)₂₈ ─ CH₃
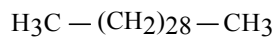
Triacontane (**101**)
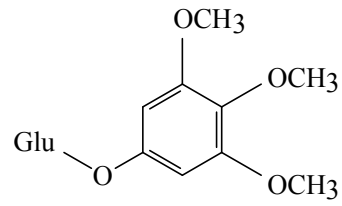
3,4,5-Trimethoxyphenol-1-*O*-β-D-glucopyranoside (**102**)
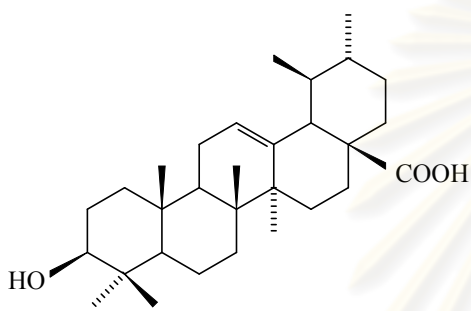
Ursolic acid (**103**)
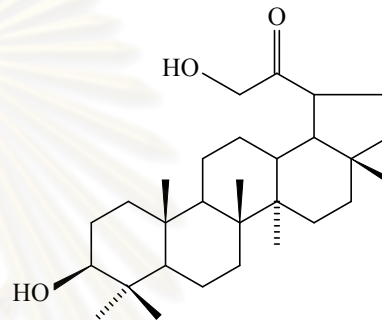
Wallichenol (**104**)

Figure 4. (continued)

CHAPTER III
EXPERIMENTAL

1. Source of Plant Material

The dried roots of *Maerua siamensis* were purchased from Vejpong Osoth, a traditional Thai pharmacy, in Bangkok, Thailand, on March 2009, and later compared with the plant samples collected from the Central Laboratory and Greenhouse Complex, Kasetsart University (Kamphaengsaen Campus), Nakorn Pathom, Thailand.

2. General Techniques

2.1 Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

2.2 Analytical Thin-Layer Chromatography (TLC)

Technique:	One dimension, ascending
Adsorbent:	Silica gel 60 F_{254} (E. Merck) pre-coated plates
Layer thickness:	0.2 mm
Distance:	5 cm
Temperature:	Laboratory temperature (30-35 ^{o}C)
Detection:	1. Ultraviolet light (254 and 365 nm)
	2. 10% Sulfuric acid and heating at 105 ^{o}C for 10 minutes

2.3 Column Chromatography

2.3.1 Conventional Column Chromatography

Absorbant:	Silica gel 60 number 9385 (particle size 0.040-0.063 nm) and number 7734 (particle size 0.063-0.200 nm) (E. Merck)
Packing method:	Wet packing: The absorbent was mixed with the eluent into slurry, then poured into a column and allowed to settle.
Sample loading:	The sample was dissolved in a small amount of the eluent, and then applied gently on top of the column.

Detection:	Fractions were examined by TLC technique in the same manner as described in section 2.2.

2.3.2 Size-Exclusion Column Chromatography

Gel filter:	Sephadex LH-20 (Pharmacia Biotech AB)
Packing method:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading:	The sample was dissolved in a small amount of eluent, and then applied gently on top of the column.
Detection:	Fractions were examined by TLC technique in the same manner as described in section 2.2.

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Spectra

UV spectra were obtained on a Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.2 Infrared (IR) Spectra

IR spectra (KBR disc) were recorded on a Perkin Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.4.3 Mass Spectra

Electrospray Ionization (ESI) mass spectra were obtained on a Micromass LCT mass spectrometer (National Center for Genetic Engineering and Biotechnology, BIOTEC, Thailand).

2.4.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C) Spectra

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker DPX-300 FT-NMR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were obtained on a JEOL JMN-A500, Varian Unity INOVA (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.5 Physical Properties

2.5.1 Melting Points

Melting points were obtained on a Fisher-John melting point apparatus (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

3. Extraction and Isolation of Compounds from the Roots of *Maerua siamensis*

3.1 Extraction of the Roots of *M. siamensis*

The dried roots of *M. siamensis* (10 kg) were ground and then macerated with methanol (5 × 30 L, 3 days each) at room temperature. The methanol filtrates were combined and evaporated under reduced pressure to give the methanol extract (700 g, 7.0% yield, based on dried weight of the roots). Water was added to the methanol extract before it was partitioned with hexane exhaustively to give the hexane extract (32 g, 0.32% yield). The residual methanol extract was further partitioned with EtOAc to give the EtOAc (45 g, 0.45% yield) and aqueous extracts (700 g, 7% yield).

Dried roots of *Maerua siamensis* (10 kg)

macerate with methanol

Methanol extract

add water / partition with hexane

Hexane extract (32 g)   Aqueous extract

partition with EtOAc

EtOAc extract (45 g)   Aqueous extract (700 g)
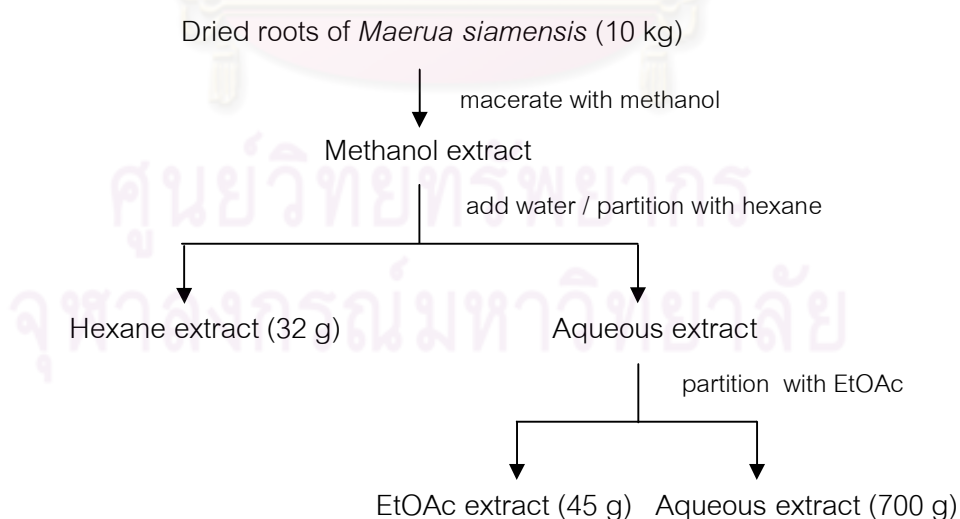
Scheme 1. Extraction of *M. siamensis* roots

3.2 Isolation of Compounds from the Hexane Extract of *M. siamensis* Roots

A portion of the hexane extract (15 g) was subjected to silica gel column chromatography. The extract was re-dissolved in a small volume of hexane and triturated with an amount of silica gel. The mixture was left to dry at room temperature, then applied to the top of a silica gel column (600 g, 9.5 × 15 cm). The column was eluted with dichloromethane (CH_2Cl_2). Two hundred and forty fractions (50 ml each) were collected and combined according to their TLC profiles (CH_2Cl_2 as the mobile phase) into 7 major fractions (MH1- MH7), as shown in **Table 4**.

Table 4. Combined fractions from the hexane extract of *M. siamensis* roots

Fraction Code	Weight (g)
MH1	1.42
MH2	0.54
MH3	0.36
MH4	0.39
MH5	2.25
MH6	1.14
MH7	8.10

3.2.1 Isolation of Compound MS-1 (β-Sitosterol)

Fraction MH5 (2.25 g) was further purified on a silica gel column (90 g, 3 × 40 cm) washed down with CH_2Cl_2. One hundred and eight collected fractions (15 ml each) were examined by TLC (using CH_2Cl_2 as the mobile phase), then combined to yield 6 subfractions (MH51-MH56). Compound MS-1 precipitated as colorless needles (330.0 mg, 0.007% yield) from subfraction MH56.

3.2.2 Isolation of Compound MS-2 (Vanillin)

Subfraction MH55 (80 mg) was separated on a silica gel column (6 g, 1.7 × 10 cm), eluted with hexane-acetone (3:1), into 36 subfractions (5 ml each). After TLC examination (mobile phase: hexane-acetone = 3:1), these subfractions were combined

into 5 major ones (MH551- MH555). Evaporation of the solvent from subfraction MH555 yielded compound MS-2 as light brown amorphous solid (7.5 mg, 0.00016% yield).

3.2.3 Isolation of Compound MS-3 (Lupeol)

Fraction MH3 (0.36 g) was separated on a silica gel column (20 g, 2.3 × 13 cm) eluted with hexane-acetone (24:1). Thirty-seven subfractions (10 ml each) were collected and pooled, after TLC monitoring (mobile phase: hexane-acetone = 24:1, 9:1), into 5 major subfractions (MH31- MH35). Subfraction MH33 (0.15 g) was chromatographed on a silica gel column (7.5 g, 1.3 × 13 cm) eluted with hexane-CH_2Cl_2 (1:3). Sixty-one subfractions (10 ml each) were collected and combined according to their TLC pattern in hexane-CH_2Cl_2 (1:3) into 5 subfractions (MH331-MH335). Subfraction MH335 (50 mg) was subjected to further purification on another silica gel column, using hexane-CH_2Cl_2 (1:3) as the eluent, to yield subfractions MH3351-MH3353. Compound MS-3 was obtained as a white powder (5.0 mg, 0.00011% yield) after removal of the organic solvent from subfraction MH3353.

3.3 Isolation of Compounds from the EtOAc Extract of *M. siamensis* Roots

A portion (40 g) of the EtOAc extract was subjected to silica gel column chromatography. The extract was re-suspended in a small amount of EtOAc and triturated with silica gel. After being left to dry at room temperature, the mixture was applied to the top of a silica gel column (600 g, 9.5 × 15 cm) and washed down with a solvent system of isocratic CH_2Cl_2-MeOH (49:1). Two hundred and sixteen fractions (50 ml each) were collected. Monitoring of their TLC profiles (mobile phase: CH_2Cl_2-MeOH = 49:1, 19:1) led to the combination of these fractions into eight major ones (ME1-ME8), as shown in **Table 5**.

3.3.1 Isolation of Compound MS-4 (7-Hydroxy-6-methoxycyclobrassinone)

Fraction ME5 (1.17 g) was further separated on a silica gel column (45 g, 2 × 20 cm), eluted with a solvent system of CH_2Cl_2-acetone (22:3), into 55 subfractions which were then pooled according to their TLC pattern (mobile phase: CH_2Cl_2-acetone = 23:2 → 9:1) into 6 major ones (ME51-ME56). Subfraction ME55 (0.47 g) was selected for chromatographic separation on a silica gel column (25 g, 2.5 × 12 cm), using CH_2Cl_2-acetone (9:1) as the eluent, to give 4 subfractions (ME551-ME554). Size-exclusion chromatography of subfraction ME553 (0.35 g) on a Sephadex LH-20 column,

Table 5. Combined fractions from the EtOAc extract of *M. siamensis* roots

Fraction Code	Weight (g)
ME1	0.04
ME2	0.16
ME3	0.35
ME4	0.20
ME5	1.17
ME6	1.37
ME7	1.02
ME8	28.41

washed down with CH_2Cl_2-MeOH (1:1), gave 6 subfractions (ME5531-ME5536). Subfraction ME5534 (100 mg), which displayed a distinct orange spot on TLC (mobile phase: CH_2Cl_2-acetone = 9:1), was further purified on another Sephadex LH-20 column eluting with CH_2Cl_2-MeOH (1:1). Compound MS-4 was obtained as an orange powder (5.2 mg) upon evaporation of the solvent from the third subfraction.

In addition, silica gel column chromatography of fraction ME6 (1.37 g) eluting with CH_2Cl_2-MeOH (24:1) gave 6 major subfractions (ME61-ME66). Repeated size-exclusion chromatography of subfraction ME63 on Sephadex LH-20 columns, each one washed down with CH_2Cl_2-MeOH (1:1), yielded an additional amount (0.5 mg) of compound MS-4. Therefore, the total amount of this compound isolated from the EtOAc extract of *M. siamensis* roots was 5.7 mg, and the total yield was 0.000064%.

3.3.2 Isolation of Compound MS-5 (7-Hydroxycyclobrassinone)

Separation of fraction ME7 (1.02 g) on a silica gel column (50 g, 2.5 × 15 cm), eluted with CH_2Cl_2-MeOH (24:1), gave 76 subfractions (15 ml each). They were examined by TLC (mobile phase: CH_2Cl_2-MeOH = 24:1 → 47:3) before being combined into 6 major subfractions (ME71-ME76). Subfraction ME74 (0.30 g) was further chromatographed on a Sephadex LH-20 column washed down with CH_2Cl-MeOH (1:1) to give 17 subfractions. These subfractions were pooled after TLC monitoring (mobile phase: CH_2Cl_2-MeOH = 47:3) into 3 subfractions (ME741-ME743). Subfraction ME743

(70 mg), which showed an orange spot on TLC (mobile phase: CH_2Cl_2-acetone = 4:1), was further separated on a Sephadex LH-20 column eluted with CH_2Cl_2-MeOH (1:1). Seven subfractions were collected, examined by TLC (mobile phase: CH_2Cl_2-MeOH = 47:3), then combined to yield subfractions ME7431-ME7433. Compound MS-5 was obtained as an orange powder (7.1 mg) upon evaporation of the solvent from subfraction ME7432.

Another subfraction, ME75 (0.32 g), was subjected to silica gel column chromatography (16 g, 1.3 × 28 cm) eluting with CH_2Cl_2-acetone (4:1) to give 26 subfractions (10 ml each). After examination by TLC (mobile phase: CH_2Cl_2-acetone = 4:1), these subfractions were combined into 3 subfractions (ME751-ME753). Subfraction ME753 (40 mg), displaying similar orange spot on TLC as above, was purified on a Sephadex LH-20 column using CH_2Cl_2-MeOH (1:1) as the mobile phase to give two subfractions (ME7531-ME7532). An additional amount of compound MS-5 (5.3 mg) was obtained upon evaporation of the solvent from subfraction ME7532. Therefore, the total yield of compound MS-5 was 12.4 mg (0.00014% yield).

Hexane extract of *M. siamensis* roots (15 g)

Silica gel CC, CH$_2$Cl$_2$

MH1   MH2   MH3   MH4   MH5   MH6   MH7

Silica gel CC, hexane-acetone (24:1)

MH31   MH32   MH33   MH34   MH35

Silica gel CC, CH$_2$Cl$_2$

MH51   MH52   MH53   MH54   MH55   MH56

Silica gel CC, hexane-CH$_2$Cl$_2$ (1:3)

MH331   MH332   MH333   MH334   MH335

Silica gel CC

hexane-acetone (3:1)

MS-1

330.0 mg

Silica gel CC, hexane-CH$_2$Cl$_2$ (1:3)

MH3351   MH3352   MH3353

MH551   MH552   MH553   MH554   MH555

MS-3

5.0 mg

MS-2

7.5 mg

Scheme 1. Extraction and isolation of compounds from the hexane extract of *M. siamensis* roots

EtOAc extract of *M. siamensis* roots (40 g)

Silica gel CC, CH_2Cl_2-MeOH (49:1)

| ME1 | ME2 | ME3 | ME4 | ME5 | ME6 | ME7 | ME8 |

Silica gel CC, CH_2Cl_2-acetone (22:3)

| ME51 | ME52 | ME53 | ME54 | ME55 | ME56 |

Silica gel CC, CH_2Cl_2-acetone (9:1)

| ME551 | ME552 | ME553 | ME554 |

Sephadex LH-20, CH_2Cl_2-MeOH (1:1)

| ME5531 | ME5532 | ME5533 | ME5534 | ME5535 | ME5536 |

Sephadex LH-20, CH_2Cl_2-MeOH (1:1)

| ME55341 | ME55342 | ME55343 | ME55344 |

MS-4

5.2 mg

Scheme 2. Extraction and isolation of compounds from the EtOAc extract of *M. siamensis* roots

ME7

Silica gel CC, CH$_2$Cl$_2$-MeOH (24:1)

ME71 ME72 ME73 ME74 ME75 ME76

Sephadex LH-20, CH$_2$Cl$_2$-MeOH (1:1)   Silica gel CC, CH$_2$Cl$_2$-acetone (4:1)

ME741 ME742 ME743 ME751 ME752 ME753

Sephadex LH-20, CH$_2$Cl$_2$-MeOH (1:1)   Sephadex LH-20, CH$_2$Cl$_2$-MeOH (1:1)

ME7431 ME7432 ME7433 ME7531 ME7532

MS-5

7.1 mg

MS-5

5.3 mg
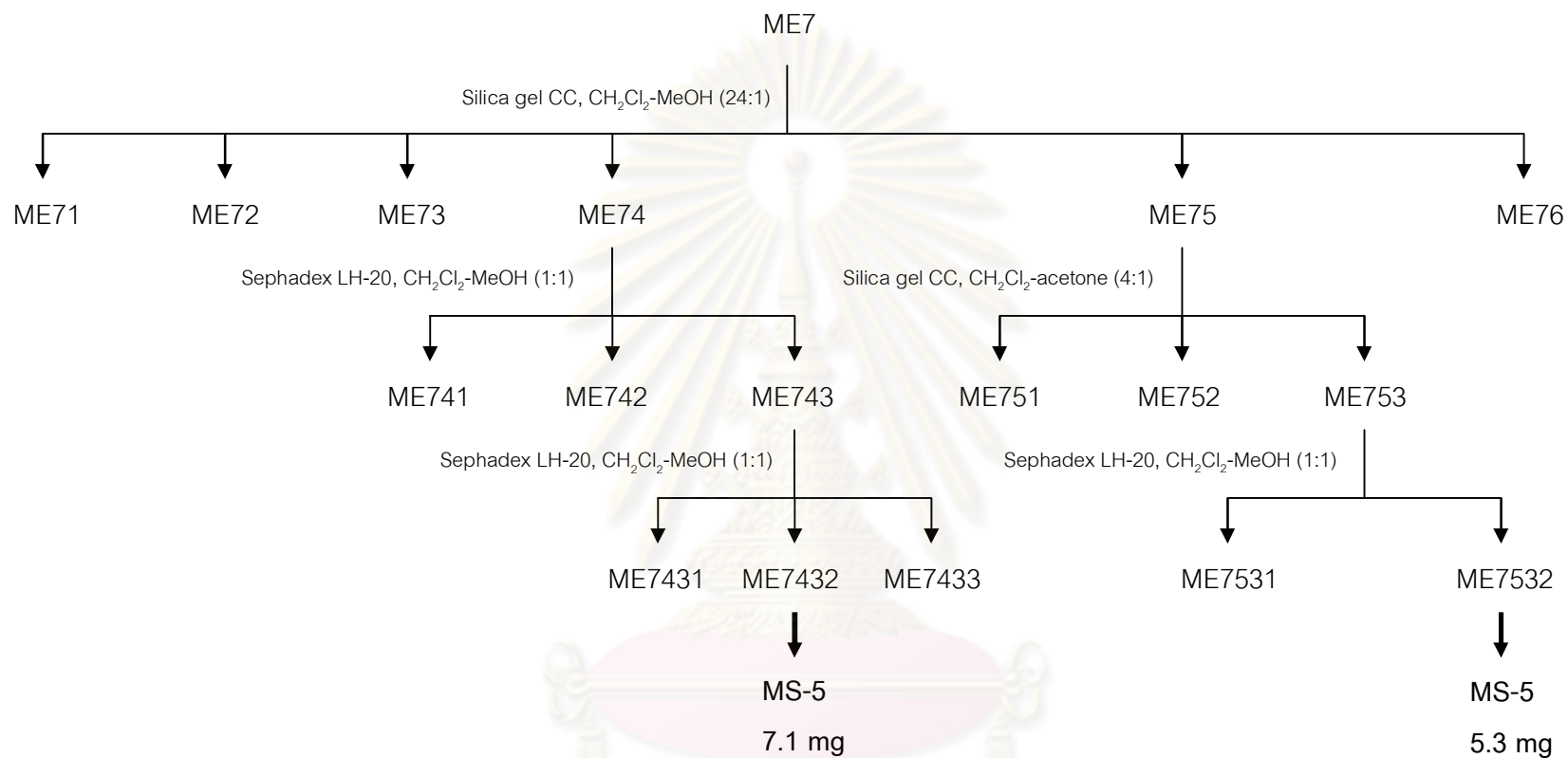
Scheme 2. (continued)

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound MS-1 (β-Sitosterol)

Compound MS-1 was obtained as colorless needles in MeOH (330.0 mg, 0.007% based on dried weight of the *M. siamensis* roots). The compound is soluble in CH_2Cl_2.

Mp:	136-138 $^{\circ}$C
ESI-MS:	*m/z* (% rel. int.): 438 [M+H+Na]$^+$ (29); **Figure 5**.
IR:	ν_{max} cm^{-1} (KBr): 3424, 2960, 1465, 1381, 1061; **Figure 6**.
^{1}H NMR:	δ ppm, 300 MHz, in CDCl$_3$; 0.66 (3H, *s*), 0.79 (3H, *d, J* = 6.6 Hz), 0.81 (3H, *d, J* = 6.6 Hz), 0.83 (3H, *t, J* = 7.0 Hz), 0.90 (3H, *d, J* = 6.6 Hz), 0.99 (3H, *s*), 3.50 (1H, *m*) and 5.33 (1H, *d, J* = 5.1 Hz); **Table 6** and **Figures 7a-7b**.
^{13}C NMR:	δ ppm, 75 MHz, in CDCl$_3$; 11.9, 12.0, 18.8, 19.0, 19.4, 19.8, 21.1, 23.1, 24.3, 26.1, 28.2, 29.2, 31.7, 31.9, 31.9, 34.0, 36.1, 36.5, 37.3, 39.7, 42.3, 42.3, 45.9, 50.1, 56.1, 56.7, 71.8, 121.7 and 140.8; **Table 6** and **Figures 8a-8b**.

4.2 Compound MS-2 (Vanillin)

Compound MS-2, which was obtained as brownish amorphous solid (7.5 mg, 0.00016% based on dried weight of the roots), is soluble in CH_2Cl_2.

Mp:	80-81°C
ESI-MS:	*m/z* (% rel. int.): 151 [M-H]$^+$ (100), 91 (41); **Figure 9**.
IR:	ν_{max} cm^{-1} (KBr): 3444, 2938, 1669, 1151, 814; **Figure 10**.
^{1}H NMR:	δ ppm, 300 MHz, in CDCl$_3$; 3.99 (3H, *s*), 6.27 (1H, *s*), 7.01 (1H, *d, J* = 8.4 Hz), 7.44 (2H, *m*) and 9.84 (1H, *s*); **Table 7** and **Figure 11**.
^{13}C NMR:	δ ppm, 75 MHz, in CDCl$_3$; 56.1, 108.8, 114.4, 127.5, 129.9, 147.2, 151.7 and 190.8; **Table 7** and **Figure 12**.

4.3 Compound MS-3 (Lupeol)

Compound MS-3 was obtained as white powder (5.0 mg, 0.00011% based on dried weight of the roots). The compound is soluble in CH_2Cl_2.

Mp:	214-215 $^{\circ}$C
ESI-MS:	m/z (% rel. int.): 449 [M+Na]$^{+}$ (20); **Figure 13**.
IR:	$\mathbf{V}_{max}$ cm^{-1} (KBr): 3433, 2942, 1454, 1380, 1043; **Figure 14**.
^{1}H NMR:	δ ppm, 300 MHz, in CDCl$_3$; 0.74 (3H, s), 0.77 (3H, s), 0.81 (3H, s), 0.92 (3H, s), 0.95 (3H, s), 1.01 (3H, s), 1.23 (3H, s), 2.35 (1H, td, J = 11.1, 5.7 Hz), 3.17 (1H, dd, J = 10.5, 5.1 Hz), 4.56 (1H, s) and 4.68 (1H, s); **Table 8** and **Figures 15a-15b**.
^{13}C NMR:	δ ppm, 75 MHz, in CDCl$_3$; 14.6, 15.4, 16.0, 16.1, 18.0, 18.3, 19.3, 21.0, 25.2, 27.4, 27.4, 28.0, 29.7, 34.3, 35.6, 37.2, 38.0, 38.7, 38.9, 40.0, 40.9, 42.8, 43.0, 48.3, 48.7, 50.5, 55.3, 79.0, 109.3 and 151.0; **Table 8** and **Figures 16a-16b**.

4.4 Compound MS-4 (7-Hydroxy-6-methoxycyclobrassinone)

Compound MS-4 was obtained as an orange powder (5.2 mg, 0.00006% based on dried weight of the roots). It is soluble in methanol.

Mp:	260-262 $^{\circ}$C (decomposed)
UV:	λ_{max} (MeOH) nm (log ε): 220 (4.24), 260 (4.08), 291 (4.35), 350 (3.68); **Figure 17**.
ESI-MS:	m/z (% rel. int.): 301 [M + Na]$^{+}$ (47.41), 279 [M + H]$^{+}$ (100); **Figure 18**.
IR:	$\mathbf{V}_{max}$ cm^{-1} (KBr): 3436, 3127, 1654, 1578, 1468, 1357, 1300, 1007; **Figure 19**.
^{1}H NMR:	δ ppm, 500 MHz, in DMSO-d_6; 3.80 (3H, s), 4.10 (3H, s), 6.91 (1H, s), 7.13 (1H, s), 9.12 (1H, s) and 12.29 (1H, $br\ s$); **Table 9** and **Figure 20**.
^{13}C NMR:	δ ppm, 125 MHz, in DMSO-d_6; 56.0, 63.9, 99.9, 101.6, 113.5, 114.7, 135.5, 144.9, 145.8, 150.5, 151.6, and 172.4; **Table 9** and **Figure 21**.

4.5 Compound MS-5 (7-Hydroxycyclobrassinone)

Compound MS-5 was obtained as an orange powder (12.4 mg, 0.00014% based on dried weight of the roots). The compound is soluble in methanol.

Mp:	265-267 $^{\circ}$C (decomposed)
UV:	λ_{max} (MeOH) nm (log ε): 220 (4.28), 258 (4.04), 288 (4.21); **Figure 24**.

ESI-MS: *m/z* (% rel. int.): 271 [M + Na]$^+$ (100), 249 [M + H]$^+$ (81.73); **Figure 25**.

IR: ν_{max} cm^{-1} (KBr): 3380, 3287, 1650, 1581, 1457, 1371, 1003; **Figure 26**.

^{1}H NMR: δ ppm, 500 MHz, in DMSO-d_6; 4.10 (3H, *s*), 6.70 (1H, *dd*, *J* = 8.5, 2.3 Hz), 6.88 (1H, *d*, *J* = 2.3 Hz), 7.44 (1H, *d*, *J* = 8.5 Hz) and 9.57 (1H, s); **Table 10** and **Figure 27**.

^{13}C NMR: δ ppm, 125 MHz, in DMSO-d_6; 63.9, 99.1, 112.2, 113.2, 115.2, 119.3, 142.4, 150.4, 153.3, 154.9, and 172.2; **Table 10** and **Figure 28**.

5. Evaluation of Biological Activities

Cytoxicity and anti-*Mycobacterium tuberculosis* activity assays were performed at the National Center for Genetic Engineering and Biotechnology, BIOTEC, Pathumthani, Thailand.

5.1 Determination of Cytotoxic Activity Against NCI-H187, KB and MCF-7 Cell Lines

The cytotoxic activity of the isolated compounds against human small cell lung carcinoma (NCI-H187, ATCC CRL-5804), epidermoid carcinoma of oral cavity (KB, ATCC CCL-17) and breast adenocarcinoma (MCF-7, ATCC HTB-22) cell lines was assayed using the method described by O'Brien *et al.* (2000). In brief, NCI-H187 and MCF-7 cells were diluted to 9×10^5 cells/ml and KB cells were diluted to 7×10^5 cells/ml in fresh medium. The cell suspensions were incubated at 37°C in 5% CO_2 incubator overnight, then the samples were added. After the incubation period (5 days for NCI-H187 and 3 days for KB and NCF-7), 12.5 µl of 62.5 µl/ml resazurin solution was added to each well, and the plates were then incubated at 37°C for 4 hours. Fluorescence signal was measured at the excitation and emission wavelengths of 530 nm and 590 nm, respectively. Percent inhibition of cell growth was calculated as follows.

$$\% \text{ Inhibition} = [1- (FUT/ FUC)] \times 100$$

whereas FUT and FUC are the mean fluorescent unit from treated and untreated conditions, respectively.

Dose response curves were plotted from 6 concentrations of 2-fold serially diluted test compounds and the sample concentrations that inhibited cell growth by 50% (IC50) were derived using the SOFTMax Pro software (Molecular Devices, USA). The

compound was considered strongly active, moderately active, weakly active or inactive if its IC_{50} value was less than 5 µl/ml, between 5-10 µl/ml, between 10-20 µl/ml or more than 20 µl/ml, respectively.

5.2 Determination of Anti-*Mycobacterium tuberculosis* Activity

Green fluorescent protein (GFP)-expressing *Mycobacterium tuberculosis* strain $H_{37}Ra$, established by Changsen *et al.* (2003), was used. The mycobacteria were cultivated on 7H10 agar containing 30 µg/ml kanamycin at $37^{o}C$ for 4 weeks or until growth was observed. Starter culture was prepared by fully looping 2-3 single colonies into 7H9 broth supplemented with 0.2% v/v glycerol, 0.1% w/v solution (BD Biosciences) and 30 µg/ml of kanamycin. The mixture was then incubated at $37^{o}C$ in 200 rpm shaker incubator until the optical density (OD) at 550 nm was between 0.5 and 1.

For batch cultivation, the starter cultures were transferred at the rate of 1/10 volume to the 7H9 broth and incubated at $37^{o}C$ in 200 rpm shaker incubator until the OD at 550 nm was approximately 0.5 to 1. The cells were pelleted, washed and suspended in PBS buffer, and then sonicated 8 times for 15 seconds each. The sonicated samples were then aliquoted and frozen at $-80^{o}C$ prior to use. Titer stocks were determined by colony forming unit (cfu) assay and the seeding density. For assay in 384-well format, the seeding was approximately 2×10^{4} to 1×10^{5} cfu/ml/well.

The assay was performed in duplicate. Each well contained 5 µl of test samples serially diluted in 5% dimethyl sulfoxide, followed by 45 µl of cell suspension prepared as described above. Plates were incubated at $37^{o}C$ for 7 days and the fluorescence signals were measured at the excitation and emission wavelengths of 485 and 535 nm. Fluorescence signals on day zero are used as background, which is used to subtract the signals on day 7. The percentage of growth inhibition is calculated from the mean of fluorescence unit of cells treated with sample (Fu_t) and untreated cells (Fu_c), as the following equation:

$$\% \text{ Inhibition} = [1- (Fu_t / Fu_c)] \times 100$$

The lowest drug concentration that inhibits cell growth by 90% is reported as the Minimum Inhibitory Concentration (MIC). Rifampicin, streptomycin, isoniazid and ofloxacin are used as positive controls, and 0.5%DMSO is used as a negative control.

CHAPTER IV

RESULTS AND DISCUSSION

Chemical constituents of the hexane and EtOAc extracts of the dried roots of *M. siamensis* were studied. Adsorption and size-exclusion chromatographic techniques were employed in order to isolate a total of five compounds (MS-1, MS-2, MS-3, MS-4, MS-5) from both extracts. Identification and structure elucidation of these compounds were achieved through spectroscopic techniques, including UV, IR, MS and NMR.

1. Identification of Compound MS-1 (β-Sitosterol)

Compound MS-1, obtained as colorless needles (330 mg, 0.007% yield), appeared as a purple spot upon spraying with 10% sulfuric acid in ethanol and heated. According to its $[M + H + Na]^+$ peak at *m/z* 438 in the mass spectrum (**Figure 5**), the compound should have the molecular formula $C_{29}H_{50}O$. Its IR absorption band at 3424 cm^{-1} (**Figure 6**) indicated the presence of hydroxyl function in the molecule. These data suggested that the compound might be a plant sterol.

The ^{1}H-NMR spectrum (**Figures 7a-7b**) of compound MS-1 exhibited six methyl signals. These signals can be categorized into those of two methyl singlets at δ 0.66 (H_3-18) and 0.99 ppm (H_3-19), three methyl doublets at δ 0.79 (*J* = 6.6 Hz, H_3-26), 0.81 (*J* = 6.6 Hz, H_3-27) and 0.90 ppm (*J* = 6.6 Hz, H_3-21), and a methyl triplet at δ 0.83 ppm (*J* = 7.0 Hz, H_3-29). These methyl signals are characteristic of a steroid nucleus. The proton spectrum also displayed an olefinic signal of a tri-substituted double bond at δ 5.33 ppm (1H, d, *J* = 5.1 Hz, H-6). A methine proton multiplet at δ 3.50 ppm (H-3) represents a proton geminal to a hydroxyl group of a 3β-hydroxy sterol.

The ^{13}C-NMR spectrum (**Figures 8a-8b**) of this compound displayed 29 carbon signals, including those of six methyl carbons at δ 11.9 (C-18), 12.0 (C-29), 18.8 (C-21), 19.0 (C-27), 19.4 (C-19) and 19.8 ppm (C-26), eleven methylene carbons at δ 21.1 (C-11), 23.1 (C-28), 24.3 (C-15), 26.1 (C-23), 28.2 (C-16), 31.7 (C-2), 31.9 (C-7), 34.0 (C-22), 37.3 (C-1), 39.7 (C-12) and 42.3 ppm (C-4), 9 methine carbon at δ 29.2 (C-25), 31.9 (C-8), 36.1 (C-20), 45.9 (C-24), 50.1 (C-9), 56.1 (C-17), 56.7 (C-14), 71.8 (C-3) and 121.7 ppm (C-6), and three quaternary carbon at δ 36.5 (C-10), 42.3 (C-13) and 140.8

ppm (C-5). The tri-substituted double bond between C-5 and C-6 is represented by the signals at δ 121.7 (C-6) and 140.8 ppm (C-5), and the hydroxy-substituted C-3 resonated at δ 71.8 ppm.

Following comparison of these spectral data, especially NMR data, with those previously reported (De-Eknamkul and Potduang, 2003), compound MS-1 could be identified as one of the most common plant sterol, β-sitosterol. The compound is very widely distributed in the plant kingdom and has previously been found in several members of the family Capparidaceae, for example, in the seeds of *Capparis decidua*, in the fruits of *C. moonii* and in the leaves of *C. sepiaria* (Mishra, Tomar and Lakra, 2007). For plants of the genus *Maerua*, the sterol has been reported as a constituent of *Maerua oblongifolia* (Abdel-Mogib, 1999) and *M. arenaria* (Ali *et al*., 2008), whereas its palmitate ester was found in the aerial parts of *M. crassifolia* growing in Egypt (Ibraheim, Ahmed and Ramadan, 2008).

Although the presence of β-sitosterol in higher plants is quite common, there have been a number of reports on its biological activities. For example, the compound, isolated from the stem of a cactus, was demonstrated to be the anti-inflammatory principle in the adjuvant-induced chronic inflammation model in mice (Park *et al*., 2001). The sterol and its glucoside were shown to be analgesic to mice in both the acetic-induced writhing test and the hot plate method. β-Sitosterol also exhibited *in vitro* anthelminthic activity against the worm *Ascaris suum* and *in vivo* antimutagenic activity by inhibiting the mutagenicity of tetracycline in mouse (Villasenor *et al*., 2002). Furthermore, the compound displayed therapeutic angiogenic effects on damaged blood vessels by enhancing new vessel formation in gerbil brains damaged by ischaemia/reperfusion (Choi *et al*., 2002). Recently, β-sitosterol was demonstrated to be chemopreventive against colon cancer in both *in vitro* and *in vivo* models (Baskar *et al*., 2010).
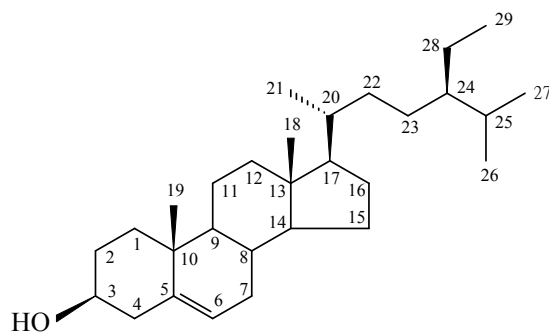
β-Sitosterol

Table 6. Comparison of the ¹H (300 MHz) and ¹³C (75 MHz) NMR spectral data of compound MS-1 and β-sitosterol (in CDCl₃).

Position	Compound MS-1		Position	β-Sitosterol	
	¹³C	¹³C		¹³C	¹³C
1	37.3	37.2	16	28.2	28.2
2	31.7	31.6	17	56.1	56.0
3	71.8	71.8	18	11.9	11.8
4	42.3	42.2	19	19.4	19.4
5	140.8	140.7	20	36.1	36.1
6	121.7	121.7	21	18.8	18.8
7	31.9	31.9	22	34.0	33.9
8	31.9	31.9	23	26.1	26.0
9	50.1	50.1	24	45.9	45.8
10	36.5	36.5	25	29.2	29.1
11	21.1	21.1	26	19.8	19.8
12	39.7	39.7	27	19.0	19.0
13	42.3	42.3	28	23.1	23.0
14	56.7	56.7	29	12.0	12.0
15	24.3	24.3			

*De-Eknamkul and Potduang, 2003

2. Identification of Compound MS-2 (Vanillin)

Compound MS-2 was obtained as light brown amorphous solid (7.5 mg, 0.00016% yield). Thin-layer chromatography of this compound gave purple color upon spraying with 10% sulfuric acid and heated. Its IR spectrum (**Figure 9**) showed absorption bands of conjugated aldehyde carbonyl at 1669 cm^{-1} and hydroxyl group at 3184 cm^{-1}. The ESI mass spectrum of compound MS-2 (**Figure 12**) displayed an [M - H]$^+$ peak at *m/z* 151, suggesting its molecular formula as $C_8H_8O_3$.

The ^{13}C-NMR spectrum (**Figure 12**) displayed eight carbon resonances, corresponding to one aldehyde carbonyl carbon at δ 190.8 ppm (C-7), one methoxyl carbon at δ 56.1 ppm (3-OCH$_3$) and six aromatic carbon signals including those of three quaternary carbons at δ 129.9 (C-1), 147.2 (C-3) and 151.7 ppm (C-4) and three methine carbons at δ 108.8 (C-2), 114.4 (C-5) and 127.5 ppm (C-6). These data indicated that compound MS-2 is an aromatic aldehyde with a hydroxyl and a methoxyl substituents.

The ^{1}H-NMR spectrum (**Figure 11**) confirmed the presence of an aldehyde function in the molecule with a one-proton singlet resonance at δ 9.84 ppm (H-7). The hydroxyl group resonated as a broad singlet at δ 6.27 ppm (4-OH), while the methoxyl group gave a three-proton singlet at δ 3.99 ppm (3-OCH$_3$). The rest of the proton resonances indicated the pattern of a 1,3,4-trisubstituted aromatic compound at δ 7.06 (*d*, *J* = 8.4 Hz, H-5), 7.44 (*dd, J* = 8.4, 1.5 Hz, H-6) and 7.44 ppm (*d, J* = 1.5 Hz, H-2).

Based on these spectroscopic data and comparison with literature values (Ito *et al.*, 2001), compound MS-2 was identified as vanillin. The compound is a well-known benzaldehyde derivative commonly used as a flavoring agent. Previously, it has been reported as a constituent of *Capparis decidua*, which is another member of the family Capparidaceae (Abdel-Mogib, Ezmirly and Basaif, 2000). Vanillin has also been found in the family Brassicaceae (Cruciferae), which is closely related to Capparidaceae. For example, it was reported as a constituent of the seeds of *Brassica juncea* (Seneviratne and Kotuwegedara, 2009).

In addition to its flavor quality, vanillin has been shown to be biologically active. The compound demonstrated potent anti-inflammatory activity through its ability to inhibit the lipopolysaccharide-stimulated activation of nuclear factor kappa B and

cyclooxygenase-2 gene expression in murine macrophage cell line (Murakami *et al*., 2007). Vanillin possesses antimicrobial potential and can be used as a natural food preservative since the compound could significantly inhibit common food pathogenic and spoilage bacteria such as *E. coli*, *S. aureus* and *B. cereus* (Fitzgerald *et al*., 2004). Vanillin also exhibited antioxidant activity by scavenging free radicals and inhibiting the photosensitization-induced lipid peroxidation and protein oxidation, preventing damage to membranes in mammalian tissues (Kamat, Ghosh and Devasagayam, 2000; Santosh Kumar, Priyadarsini and Sainis, 2002). The aromatic aldehyde could act as an anticlastogenic agent, protecting against chromosomal damage by suppressing both UV- and X-ray-induced chromosome aberrations in mammalian cells (Sasaki *et al*., 1990; Keshava *et al*., 1998). Its antimutagenic effect against spontaneous mutagenesis in *E. coli* cells might involve its ability to produce a type of DNA damage that could cause recombinational repair on damage produced by the compound itself and other DNA damage (Shaughnessy *et al*., 2006). Furthermore, it displayed chemopreventive effect in multi-organ carcinogenesis and hepatocarcinogenesis models in rats (**Tsuda** *et al*., 1994; Akagi *et al*., 1995). Vanillin also inhibited the invasion and migration of mouse breast cancer cells, suppressed the enzyme activity of matrix metalloproteinase-9, and reduced the number of lung metastasized colonies in mice. The natural compound therefore has anti-metastatic potential by decreasing invasiveness of cancer cells (Lirdprapamongkol *et al*., 2005).
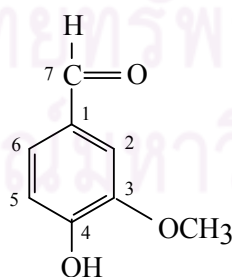
Vanillin

Table 7. Comparison of the ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compound MS-2 and vanillin (in $CDCl_3$)

Position	Compound MS-2		Vanillin*	
	^{13}C	^{1}H	^{13}C	^{1}H
1	129.9	-	129.9	-
2	108.8	7.44 (*d, J* = 1.5 Hz)	108.7	7.41 (*d, J* = 1.6 Hz)
3	147.2	-	147.1	-
4	151.7	-	151.6	-
5	114.4	7.06 (*d, J* = 8.4 Hz)	114.4	7.02 (*d, J* = 8.5 Hz)
6	127.5	7.44 (*dd, J* = 8.4, 1.5 Hz)	127.5	7.41 (*dd, J* = 8.5, 1.6 Hz)
7	190.8	9.84 (*s*)	190.9	9.81 (*s*)
3-OCH$_3$	56.1	3.99 (*s*)	56.1	3.95 (*s*)
4-OH	-	6.27 (*br s*)	-	6.19 (*s*)

*Ito *et al*., 2001

3. Identification of Compound MS-3 (Lupeol)

Compound MS-3, obtained as colorless needles (5 mg, 0.00011% yield), gave purple color on TLC after being sprayed with 10% sulfuric acid reagent and heated. IR absorption band of the compound at 3433 cm^{-1} (**Figure 13**) indicated the presence of hydroxyl substituent within the molecule. The molecular formula of $C_{30}H_{50}O$ was suggested according to its pseudomolecular [M + Na]$^+$ peak at *m/z* 449 in the ESI mass spectrum (**Figure**) and the number of carbon signals in its ^{13}C-NMR spectrum (**Figure 14**). Compound MS-3 could therefore be a pentacyclic triterpene alcohol.

The ^{1}H-NMR spectrum (**Figure 15a-15b**) of compound MS-3 exhibited a pair of one-proton broad singlets at δ 4.55 and 4.66 ppm (H_2-29) typical of the exomethylene protons in the isopropenyl group of a lupane-type triterpenoid. Other prominent peaks in the upfield region of its proton NMR spectrum are those of seven methyl singlets resonating at δ 0.74 (H_3-28), 0.77 (H_3-24), 0.81 (H_3-25), 0.95 (H_3-23 and H_3-27), 1.01 (H_3-26) and 1.66 ppm (H_3-30), respectively. A doublet of doublets at δ 3.17 ppm (1H, *J* = 10.5, 5.1 Hz) could be assigned to the methine proton (H-3) geminal to the β-hydroxyl substituent on ring A of the lupane skeleton.

The ^{13}C-NMR spectrum displayed 30 carbon resonances including those of seven methyl carbons at δ 14.6 (C-27), 15.4 (C-24), 16.0 (C-26), 16.1 (C-25), 18.0 (C-28), 19.3 (C-30) and 28.0 ppm (C-23). A pair of olefinic carbon signals at δ 109.3 (C-29) and 151.0 ppm (C-20) confirmed the presence of an exomethylene moiety in the side chain. The hydroxy-substituted methine carbon of position 3 gave a signal at δ 79.0 ppm. Comparison of these NMR data with previously published ones (Ahmad, Bano and Mohammad, 1985; Jamal, Yaacob and Din, 2008) established the identity of compound MS-3 as the lupane-type triterpenoid lupeol [lup-20(29)-en-3β-ol].

Lupeol has been reported as a constituent of various plants and has been demonstrated to possess several interesting biological activities including anti-inflammatory, chemopreventive, anti-neoplastic, cardioprotective, hepatoprotective, anti-urolithiatic, gastroprotective and wound healing properties. The triterpenoid, isolated from the stem bark of *Crataeva nurvala* (Capparidaceae), produced a reduction in rat paw swelling in adjuvant arthritis comparable to indomethacin (Geetha and Varalakshmi, 2001). Its anti-inflammatory activity might depend on its ability to prevent the production

of some pro-inflammatory mediators (Fernandez *et al*., 2001) or to suppress the immune system (Bani *et al*., 2006). Lupeol has been shown to be chemopreventive and several highly active derivatives have been developed from this triterpenoid as potential anti-neoplastic agents (Chaturvedi, Bhui and Shukla, 2008). The compound was able to modulate the role of nuclear factor kappa B and phosphatidyl inositol 3-kinase/Akt signaling pathways and inhibit skin cancer in mice (Saleem *et al*., 2004). It could also induce apoptotic death of human pancreatic adenocarcinoma cells via inhibition of Ras signaling pathway (Saleem *et al*., 2005), inhibit growth of human metastatic melanoma cells both *in vitro* and *in vivo* (Saleem *et al*., 2008) and inhibit proliferation of human prostate cancer cells by targeting β-catenin signaling (Saleem *et al*., 2009). In addition, lupeol was demonstrated to be selective catalytic inhibitor of human DNA topoisomerase II activity, with an IC_{50} value of 10.4 μM (Wada, Iida and Tanaka, 2001), and to exhibit significant antiangiogenic activity on *in vitro* tube formation of human umbilical venous endothelial cells (You *et al*., 2003).

Oral administration of lupeol to rats exerted hepatoprotective effect by scavenging the cadmium-induced free radicals and by improving the antioxidant status of the liver (Sunitha, Nagaraj and Varalakshmi, 2001). It could also revert aflatoxin B_1-induced peroxidative hepatic damage in the same animals (Preetha *et al*., 2006). Lupeol and its linoleate ester exhibited cardioprotective effect against cyclophosphamide-induced mitochondrial cardiomyopathy in male albino Wistar rats by restoration of mitochondrial structure and function (Sudharsan *et al*., 2005; 2006). The compound, administered orally at doses of 3-30 mg/kg, significantly and dose-dependently alleviated the ethanol-induced gastric damage in mice (de S. Lira *et al*., 2009). Lupeol also exhibited wound healing activity by a number of wound models (Harish *et al*., 2008) and was able to prevent the formation of the urinary gallstone in addition to reducing the size of the preformed stones in mice (Anand *et al*., 1994).
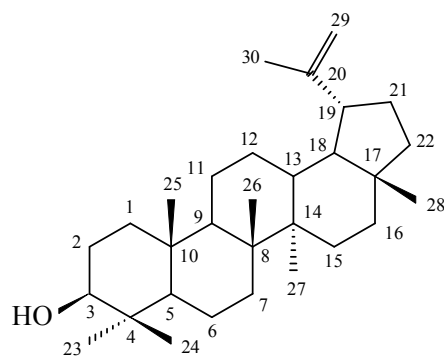
Lupeol

Table 8. Comparison of the ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compound MS-3 and lupeol (in CDCl$_3$).

Position	MS-3		Lupeol[*]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	38.7	-	38.7	-
2	27.4	-	27.4	
3	79.0	3.17 (*dd, J* = 10.5, 5.1 Hz)	78.8	3.20 (*dd, J* = 10.6 Hz)
4	38.9	-	38.8	-
5	55.3	-	55.2	-
6	18.3	-	18.3	-
7	34.3	-	34.2	-
8	40.9	-	40.8	-
9	50.5	-	50.4	-
10	37.2	-	37.1	-
11	21.0	-	20.9	-
12	25.2	-	25.1	-
13	38.0	-	38.0	-
14	42.8	-	42.9	-
15	27.4	-	27.4	-

Position	MS-3		Lupeol*	
	^{13}C	^{1}H	^{13}C	^{1}H
16	35.6	-	35.5	-
17	43.0	-	42.9	-
18	48.3	-	48.2	-
19	48.0	2.35 (*td*, *J* = 11.1, 5.7 Hz)	47.9	2.38 (*ddd*, *J* = 10.6, 10.6, 5.3 Hz)
20	151.0	-	150.6	-
21	29.7	-	29.8	-
22	40.0	-	39.9	-
23	28.0	0.95 (*s*)	28.0	0.94 (*s*)
24	15.4	0.77 (*s*)	15.4	0.76 (*s*)
25	16.1	0.81 (*s*)	16.1	0.83 (*s*)
26	16.0	1.01 (*s*)	15.9	1.03 (*s*)
27	14.6	0.95 (*s*)	14.5	0.96 (*s*)
28	18.0	0.74 (*s*)	18.0	0.79 (*s*)
29	109.3	4.55 (*br s*), 4.66 (*br s*)	109.2	4.57 (*d*, *J* = 1.0 Hz), 4.68 (*d*, *J* = 1.0 Hz)
30	19.3	1.66 (*s*)	19.3	1.67 (*s*)

*Ahmad *et al.*,1985

4. Structure Elucidation of Compound MS-4 (7-Hydroxy-6-methoxycyclobrassinone)

The IR spectrum (**Figure 19**) of the orange-color compound MS-4 (5.2 mg, 0.000064% yield) displayed a very prominent absorption band at 1654 cm^{-1}, suggesting the presence of tertiary amide carbonyl in the molecule, while another band at 3436 cm^{-1} represents the stretching of both the indole N-H and O-H bonds (Williams and Fleming, 1987). Its ^{1}H-NMR spectrum (**Figure 20**) exhibited six resonances integrated for 10 protons, whereas its ^{13}C-NMR spectrum (**Figure 21**) exhibited twelve carbon resonances including those of 2 methoxyl carbons and 1 carbonyl carbon. HSQC experiment (**Figure 22**) shows correlations between these ^{1}H- and ^{13}C-NMR peaks. From these data and its pseudomolecular [M + Na]$^{+}$ peak in the ESI mass spectrum (**Figure 18**) at *m/z* 301, the

presence of a sulfur atom in the molecular structure of compound MS-4 could be inferred and its molecular formula should therefore be $C_{12}H_{10}N_2SO_4$.

Two methoxyl singlets could be observed in its ^{1}H-NMR spectrum at δ 3.80 (6-OCH$_3$) and 4.10 ppm (2-OCH$_3$). The proton spectrum also exhibited two singlet resonances of a 1,2,4,5-tetrasubstituted aromatic ring at δ 6.91 (H-8) and 7.13 ppm (H-5), a hydroxyl proton signal at δ 9.12 ppm (7-OH) and an indole N-H resonance as a broad singlet at δ 12.29 ppm.

Twelve carbon signals of this compound could be differentiated into those of an amide carbonyl at δ 172.4 ppm (C-4), two methoxyl carbons at δ 56.0 (6-OCH$_3$) and 63.9 ppm (2-OCH$_3$), two aromatic methine carbons at δ 99.9 (C-8) and 101.6 ppm (C-5) and seven quaternary carbons at δ 113.5 (C-4a), 114.7 (C-4b), 135.5 (C-8a), 145.8 (C-6), 144.9 (C-7), 150.5 (C-2) and 151.6 ppm (C-9a). The tetrasubstituted benzene ring is a part of the indole nucleus, with a methoxyl substitution at C-6 as confirmed by a HMBC (**Figure 23b**) cross peak from the signal of methoxyl protons at δ 3.80 ppm to C-6 signal (δ 145.8 ppm). A hydroxyl substituent could be located at C-7, according to three-bond HMBC (**Figure 23d**) correlations from the hydroxyl proton signal at δ 9.12 ppm to the resonances of both C-6 and C-8 (δ 99.9 ppm). This substitution pattern was also confirmed by the HMBC (**Figure 23c and 23e**) cross peaks from H-5 signal at δ 7.13 ppm to those of C-4a (δ 113.5 ppm), C-7 (δ 144.9 ppm), C-8a (δ 135.5 ppm) and C-6 (δ 145.8 ppm), as well as from H-8 signal at δ 6.91 ppm to the carbon peaks of C-4b (δ 114.7 ppm), C-6, C-8a and C-7. Therefore, compound MS-4 possesses the rare chemical skeleton of 1,3-thiazino[6,5-*b*]indol-4-one derivative with 6-methoxy and 7-hydroxy substituents. Another methoxyl group could be located at position 2 according to a HMBC cross peak between its proton signal at δ 4.10 ppm and the signal of C-2 (δ 150.5 ppm). An indole derivative, minus the substituents at positions 6 and 7, has been named cyclobrassinone (**18**) (Gross, Porzel and Schmidt, 1994). The structure of compound MS-4 was therefore elucidated as 7-hydroxy-2,6-dimethoxy-1,3-thiazino[6,5-*b*]indol-4-one and named 7-hydroxy-6-methoxycyclobrassinone.

Although cyclobrassinone has been reported as an antifungal phytoalexin elicited by UV-irradiation of the stem tubers of kohlrabi (*Brassica oleracea* var. *gongylodes*, family Brassicaceae) (Gross *et al.*, 1994) and its synthesis has been

attempted (Suchý *et al.*, 2001), the proposed structure of this compound was later shown to be incorrect and was revised to be identical with that of rutalexin (**48**), the cruciferous phytoalexin produced by both *Brassica napus* ssp. *rapifera* and *B. oleracea* var. *gongylodes* (Pedras, Montaut and Suchý, 2004). The cyclobrassinone structure has thus never been found in nature and this is the first report of its naturally occurring derivative.

Phytoalexins found in plants of the family Brassicaceae were the first to be reported as sulfur-containing (Pedras *et al.*, 2000). The plant family is closely related to Capparidaceae and their relationship has been confirmed on the basis of the presence of glucosinolates and genetic evidences such as the DNA sequences of the rbcl gene (Fahey, Zalcmann and Talalay, 2001; Marzouk *et al.*, 2010). Hydrolysis of glucosinolates yields isothiocyanates; both of which contain sulfur atom in their molecules. Furthermore, sulfur-containing indole alkaloids have previously been isolated from a capparidaceous plant, *Capparis himalayensis* (Li *et al.*, 2008).

The biogenetic pathway of 7-hydroxy-6-methoxycyclobrassinone might follow the one proposed for cyclobrassinin (**16**). The amino acid L-tryptophan, biosynthesized from anthranilic acid via shikimate pathway, has been demonstrated to be the biogenetic precursor of most of the indole phytoalexins. The biosynthetic pathway of cyclobrassinin involves the addition of a sulfur atom from L-cysteine and a methyl group from L-methionine. Transient formation of indol-3-ylmethyl isothiocyanate was suggested as a reaction intermediate (Pedras *et al.*, 2000). Oxidation at position 4 could then yield the structure of cyclobrassinone. Further oxidation and methylation at positions 6 and 7 would give this compound.
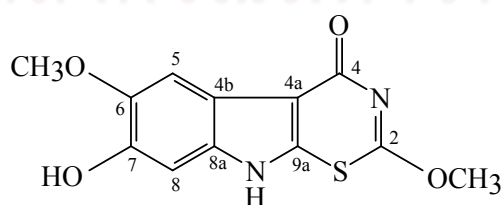
7-hydroxy-6-methoxycyclobrassinone

Table 9. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of 7-hydroxy-6-methoxycyclobrassinone (in DMSO-d_6)

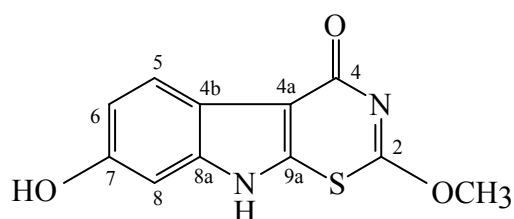| Position	^{13}C	^{1}H	HMBC
2	150.5	-	-
4	172.4	-	-
4a	113.5	-	-
4b	114.7	-	-
5	101.6	7.13 (*s*)	C-4a, C-7, C-8a, C-6
6	145.8	-	-
7	144.9	-	-
8	99.9	6.91 (*s*)	C-4b, C-6, C-8a, C-7
8a	135.5	-	-
9a	151.6	-	-
NH	-	12.29 (*br s*)	-
2-OCH$_3$	63.9	4.10 (*s*)	C-2
6-OCH$_3$	56.0	3.80 (*s*)	C-6
7-OH	-	9.12 (*s*)	C-6, C-8

5. Structure Elucidation of Compound MS-5 (7-Hydroxycyclobrassinone)

Compound MS-5 was another orange-color constituent isolated as amorphous powder (12.4 mg, 0.00014% yield) from the roots of *M. siamensis*. Its IR spectrum (**Figure 26**) is similar to that of compound MS-4, showing O-H and N-H stretching bands at 3380 cm^{-1} and a tertiary amide carbonyl band at 1650 cm^{-1}. Its ^{1}H-NMR spectrum (**Figure 27**) displayed one less methoxyl singlet than that of the previous compound. The number of carbon signals in its ^{13}C-NMR spectrum (**Figure 28**) is eleven, corresponding to the loss of one methoxyl substituent from the molecular structure of compound MS-4. This is also supported by its pseudomolecular [M + Na]$^+$ peak at *m/z* 271 in its ESI mass spectrum (**Figure 25**), indicating the molecular formula of compound MS-5 as C$_{11}$H$_8$N$_2$SO$_3$.

The ^{1}H-NMR spectrum exhibited resonances of a methoxyl group at δ 4.10 ppm (s, 2-OCH$_3$) and a hydroxyl proton as a broad singlet at δ 9.57 ppm (7-OH). Another major difference from previous compound is the set of 3 one-proton signals at δ 6.70 (dd, J = 8.5, 2.3 Hz, H-6), 6.88 (d, J = 2.3 Hz, H-8) and 7.44 ppm (d, J = 8.5 Hz, H-5), representing the 1,2,4-trisubstituted aromatic ring of the indole nucleus.

The ^{13}C-NMR spectrum displayed one methoxyl carbon signal at δ 63.9 ppm (2-OCH$_3$), three aromatic methine signals at δ 99.1 (C-8), 112.2 (C-6) and 119.3 ppm (C-5), and seven quaternary carbon signals at δ 113.2 (C-4a), 115.2 (C-4b), 142.4 (C-8a), 150.4 (C-2), 153.3 (C-9a), 154.9 (C-7) and 172.2 ppm (C-4). The carbon resonances of C-2, C-4 and 2-OCH$_3$ are nearly identical to those of compound MS-4, indicating this part of both molecules to be the same. HSQC experiment (**Figure 29**) displays correlated peaks between the proton and carbon signals of positions 5, 6, 8 and 2-OCH$_3$. A long-range HMBC (**Figure 30d**) correlation between the methoxyl proton signal and C-2 resonance could also be observed. The hydroxyl substitution at position 7 was confirmed by the HMBC (**Figure 30b and 30e**) cross peaks between this hydroxyl proton signal at δ 9.57 ppm and C-6, C-7 and C-8 resonances at δ 112.2, 154.9 and 99.1 ppm, respectively. A NOESY cross-between the resonances of this hydroxyl proton at δ 9.57 ppm and both H-6 (δ 6.70 ppm) and H-8 (δ 6.88 ppm) (**Figure 32**) also confirmed this position of the hydroxyl substitution on the ring. Therefore, the molecular structure of MS-5 was established as 7-hydroxy-2-methoxy-1,3-thiazino[6,5-b]indol-4-one and named 7-hydroxycyclobrassinone.

Although a number of alkaloids have been isolated from members of the family Capparidaceae, only a limited number of them are indole compounds. Both 7-hydroxy-6-methoxycyclobrassinone and 7-hydroxycyclobrassinone are the first indole derivatives to be reported as constituents of Maerua species of this plant family.

7-hydroxycyclobrassinone

Table 10. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of compound 7-hydroxycyclobrassinone (in DMSO-d_6)

Position	^{13}C	^{1}H	HMBC
2	150.4	-	-
4	172.2	-	-
4a	113.2	-	-
4b	115.2	-	-
5	119.3	7.44 (d, J = 8.5 Hz)	C-4a, C-4b, C-7, C-8a
6	112.2	6.70 (dd, J = 8.5, 2.3 Hz)	C-4b, C-7, C-8
7	154.9	-	-
8	99.1	6.88 (d, J = 2.3 Hz)	C-4b, C-6, C-7, C-8a
8a	142.4	-	-
9a	153.3	-	-
2-OCH$_3$	63.9	4.10 (s)	C-2
7-OH	-	9.57 (s)	C-6, C-7, C-8

6. Cytotoxicity and Anti-tuberculosis Activity of 7-Hydroxy-6-methoxycyclobrassinone and 7-Hydroxycyclobrassinone

Among the three cancer cell lines tested (NCI-H187, KB and MCF-7), the indole derivatives 7-hydroxy-6-methoxycyclobrassinone and 7-hydroxycyclobrassinone showed selective cytotoxic activity against the human small-cell lung cancer (NCI-H187) cell line only (**Table 11**). 7-Hydroxy-6-methoxycyclobrassinone was strongly active against the cancer cells (IC$_{50}$ = 1.51 μg /ml), whereas 7-hydroxycyclobrassinone was moderately active (IC$_{50}$ = 8.31 μg /ml). It is interesting to note that the structures of both compounds, especially the linear indole ring system, are similar to that of ellipticine which was used as a positive control and exhibited cytotoxicity against NCI-H187 cell line with an IC$_{50}$ value of 1.39 μg /ml.

When evaluated for their anti-tuberculosis activity against *Mycobacterium tuberculosis*, only 7-hydroxy-6-methoxycyclobrassinone was active, with an MIC of 25

µg /ml. The presence of a methoxyl substitution at position 6 of these indole derivatives therefore appears to be important for these bioactivities.

Table 11. Cytotoxicity and anti-tuberculosis activity of 7-hydroxy-6-methoxycyclobrassinone and 7-hydroxycyclobrassinone

Compound	Cytotoxicity (IC_{50}*)			Anti-TB
	NCI-H187	KB	MCF-7	(MIC*)
7-Hydroxy-6-methoxy-cyclobrassinone	1.51	Inactive	Inactive	25
7-Hydroxycyclobrassinone	8.31	Inactive	Inactive	Inactive
Ellipticine	1.39	1.14	4.03	-
Doxorubicin	0.07	0.35	9.65	-
Rifampicin	-	-	-	0.02
Isoniazid	-	-	-	0.04
Streptomycin	-	-	-	0.24
Ofloxacin	-	-	-	0.59

* in µg /ml

CHAPTER V
CONCLUSION

Phytochemical investigation of the roots of *Maerua siamensis* (Capparidaceae), which is used in traditional Thai medicine, led to the isolation of five chemical constituents, two of which are new compounds. Three known compounds (β-sitosterol, vanillin and lupeol) were isolated from the hexane extract of the roots, whereas two new indole alkaloids named 7-hydroxy-6-methoxycyclobrassinone and 7-hydroxycyclobrassinone were obtained from the EtOAc extract of the plant part. Both indole alkaloids exhibited selective cytotoxic activity against the human small-cell lung cancer (NCI-H187) cell line. 7-Hydroxy-6-methoxycyclobrassinone was strongly active against the cancer cells, while 7-hydroxycyclobrassinone was moderately active. 7-Hydroxy-6-methoxycyclobrassinone was also active when assayed against the tuberculosis-causing *Mycobacterium tuberculosis*. Both alkaloids contain a sulfur atom in their structures similar to indole phytoalexins found in plants of the closely related family Brassicaceae.

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APPENDIX
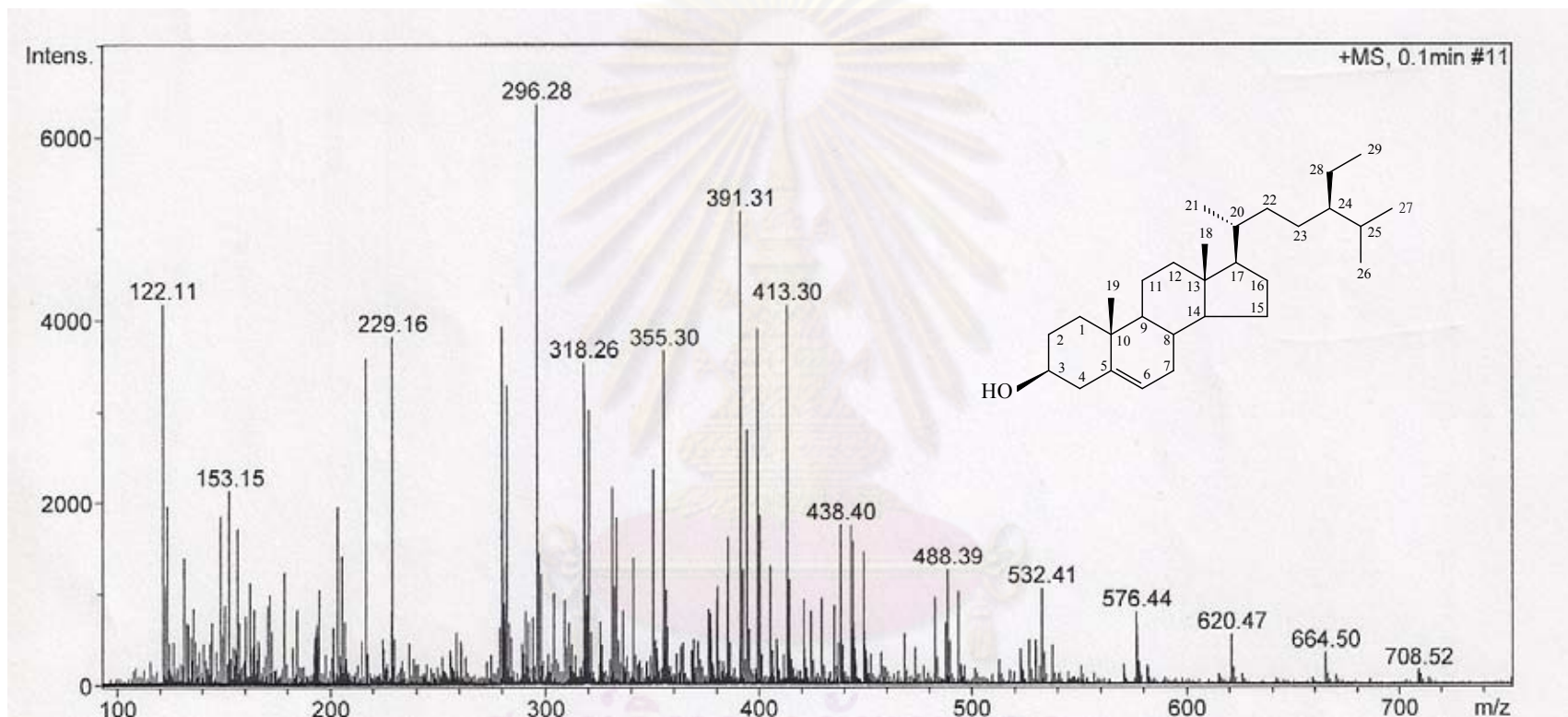
Figure 5. ESI Mass spectrum of compound MS-1
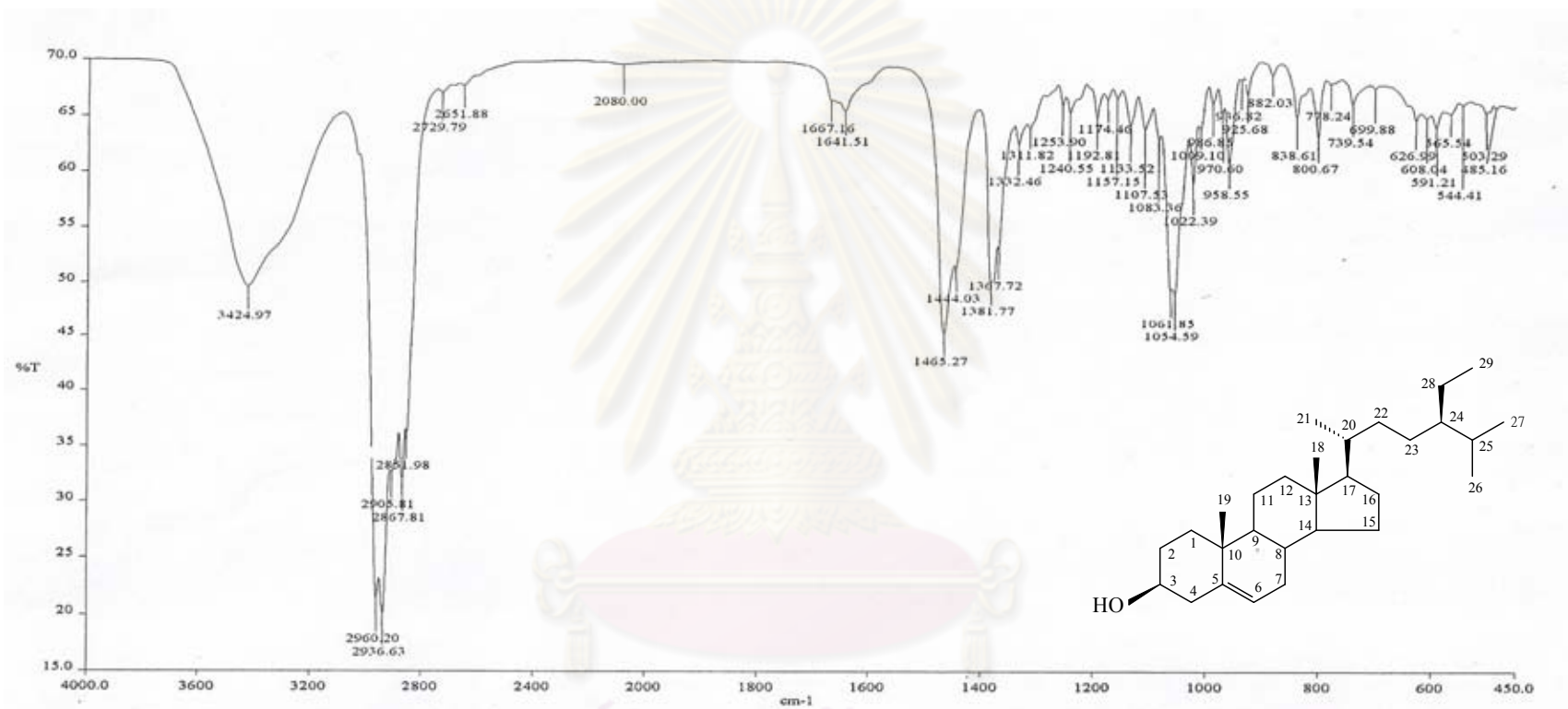
Figure 6. IR Spectrum of compound MS-1 (KBr)
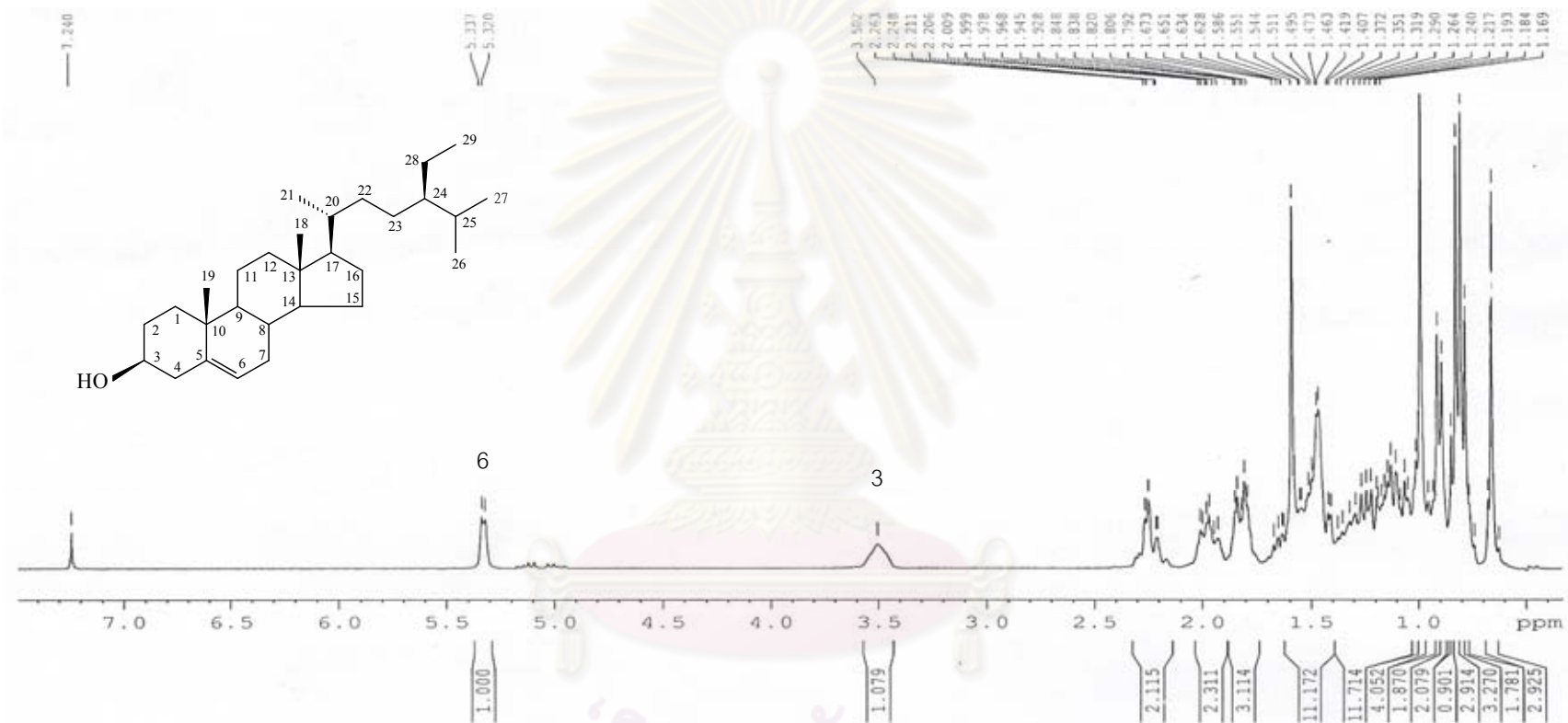
Figure 7a. ^{1}H NMR (300 MHz) Spectrum of compound MS-1 (in CDCl$_3$)
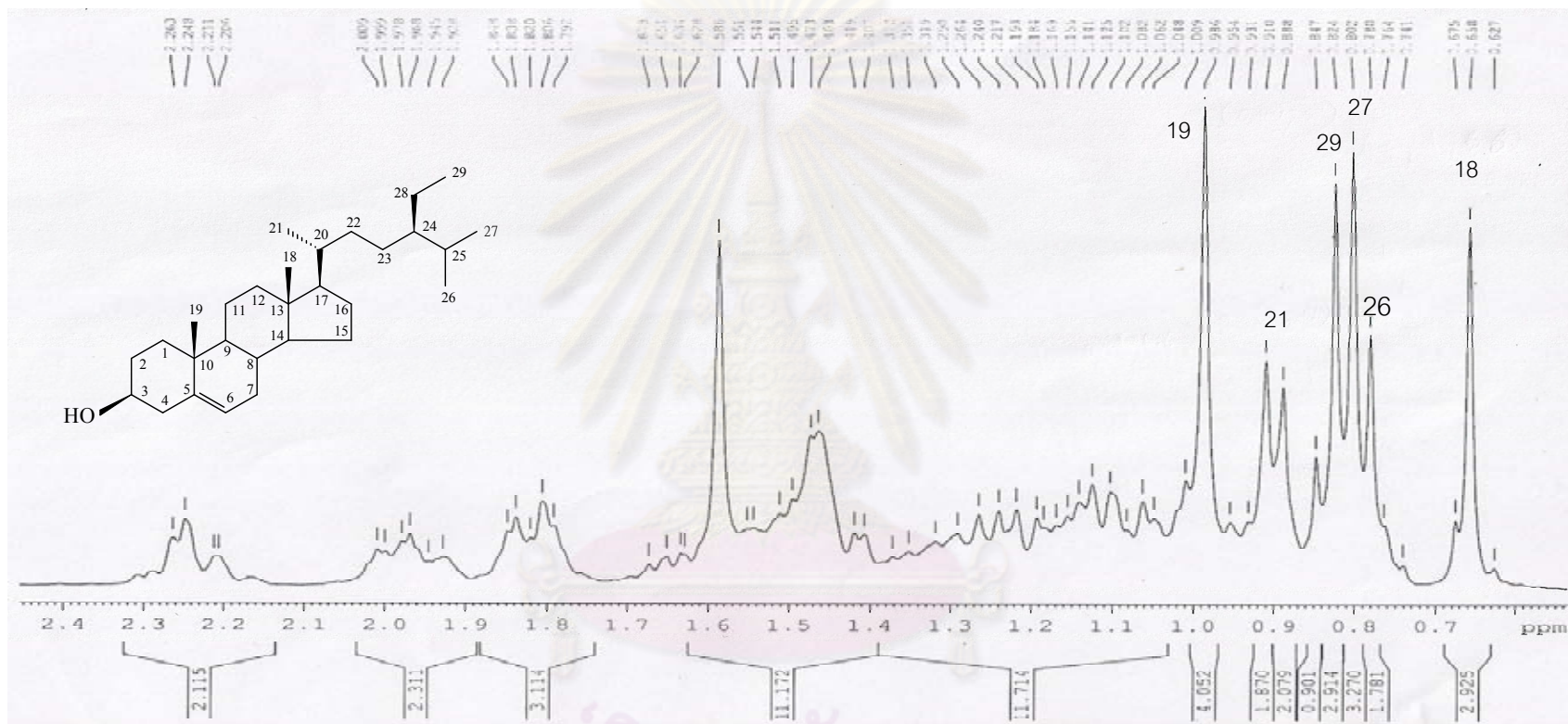
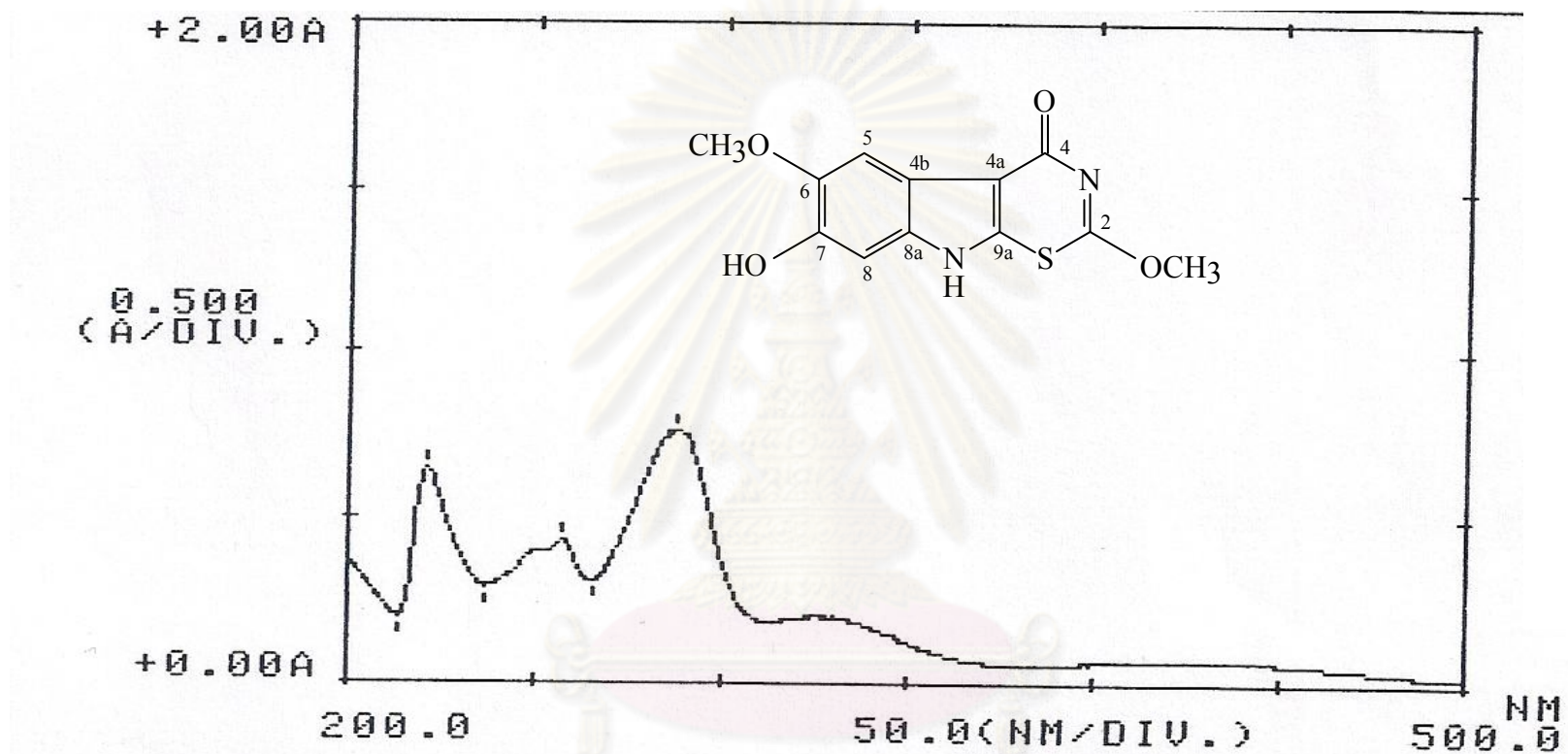**Figure 7b.** ^{1}H NMR (300 MHz) Spectrum of compound MS-1 (in CDCl$_3$) (expansion between δ 0.6-2.4 ppm)
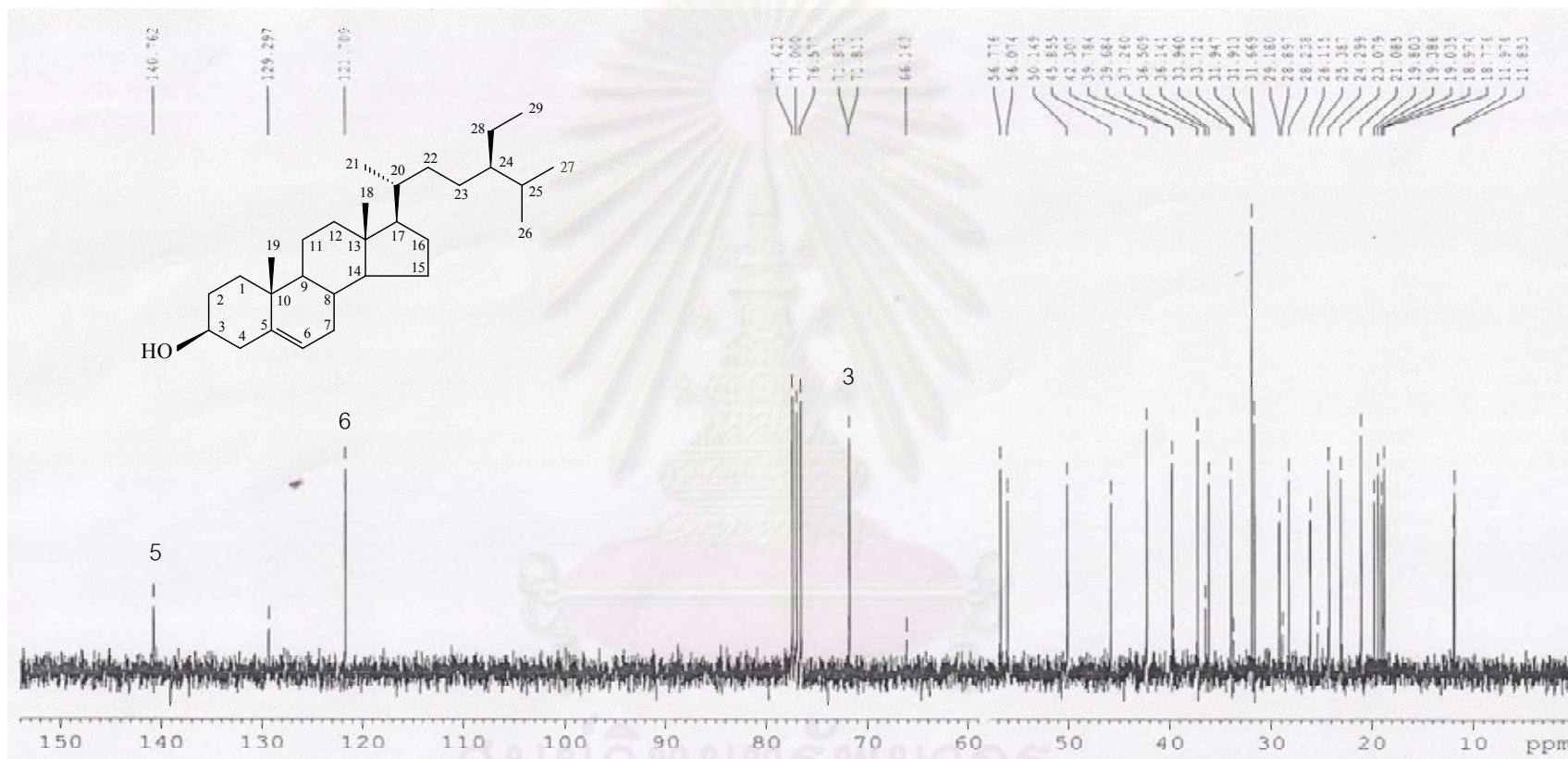
Figure 8a. ^{13}C NMR (75 MHz) Spectrum of compound MS-1 (in CDCl$_3$)
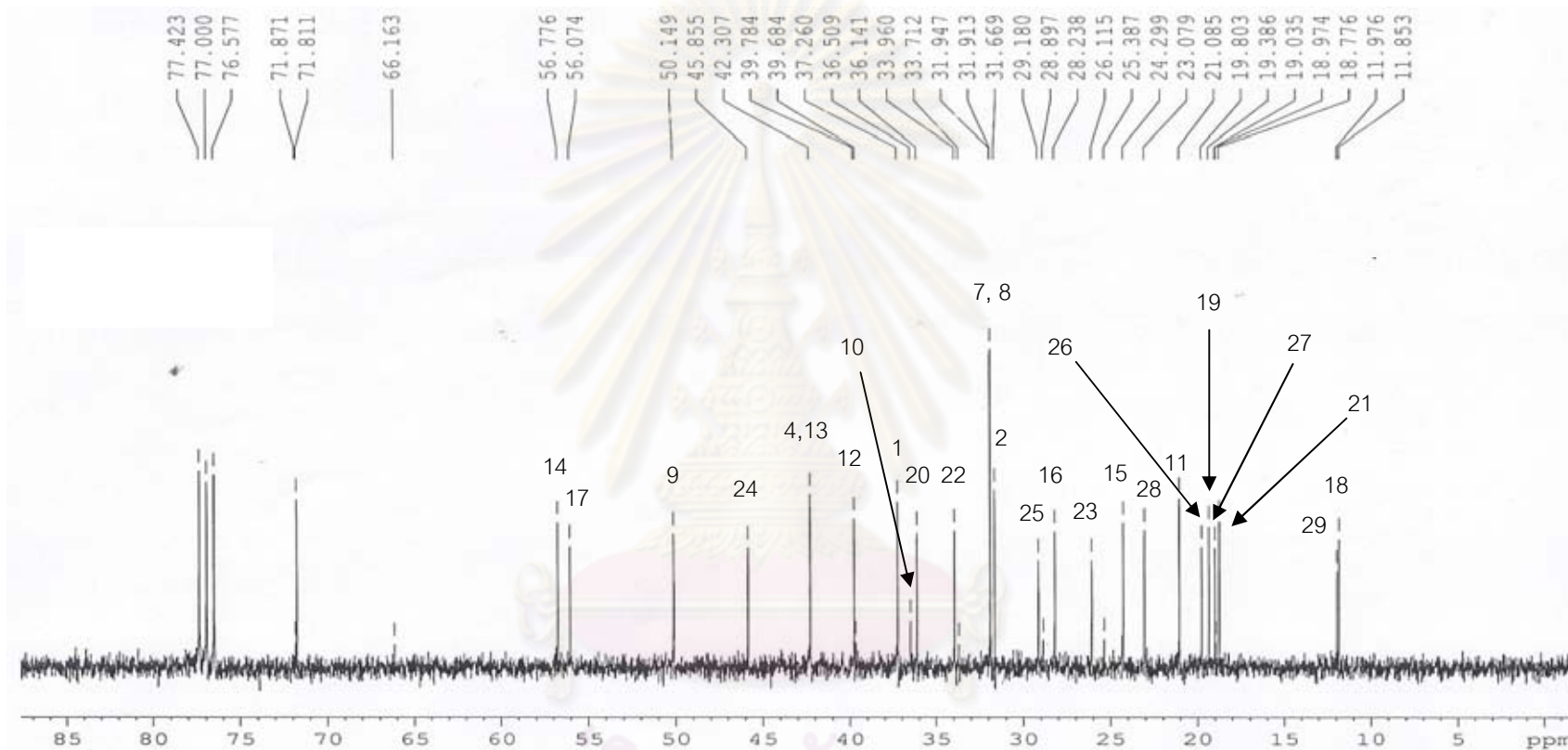
Figure 8b. ^{13}C NMR (75 MHz) Spectrum of compound MS-1 (in CDCl$_3$) (expansion between δ 0-85 ppm)
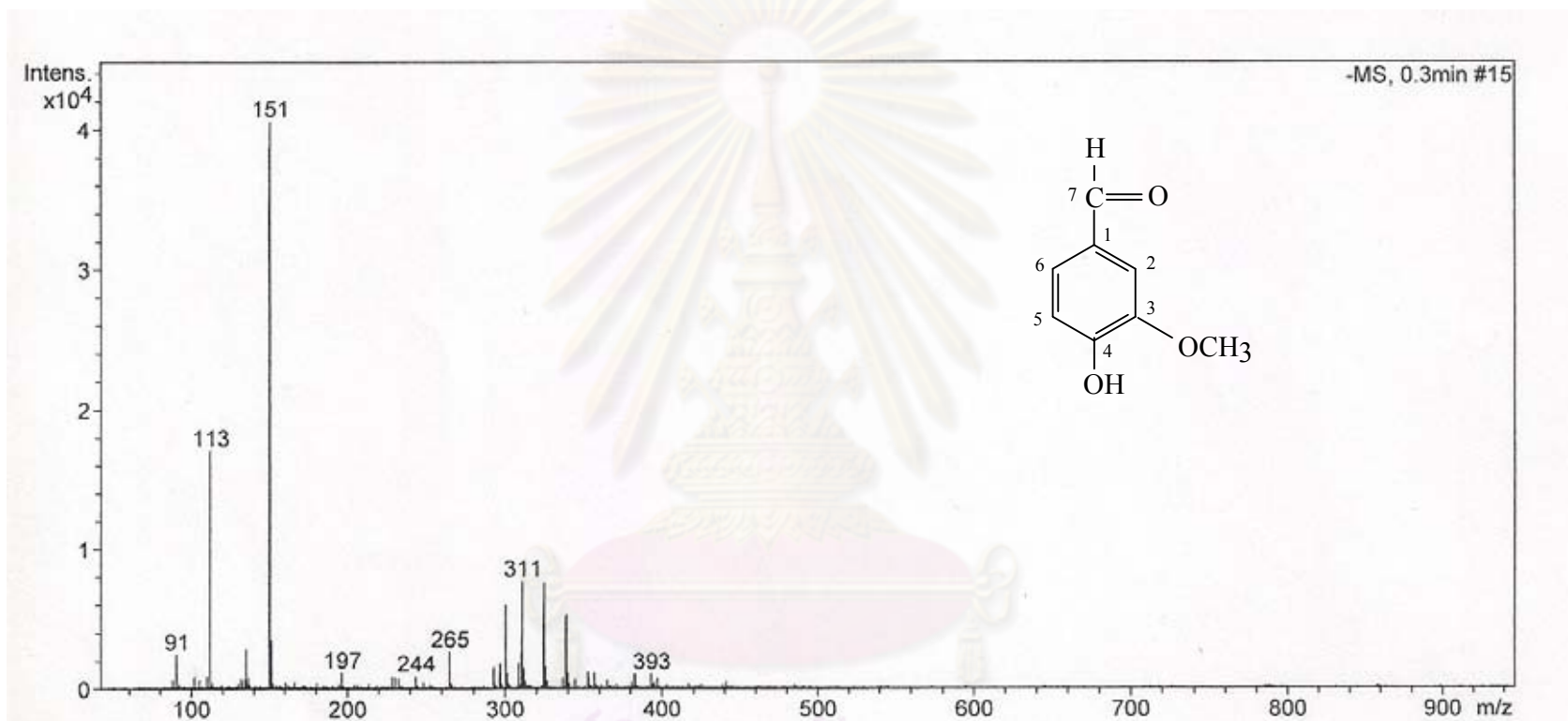
Figure 9. ESI Mass spectrum of compound MS-2
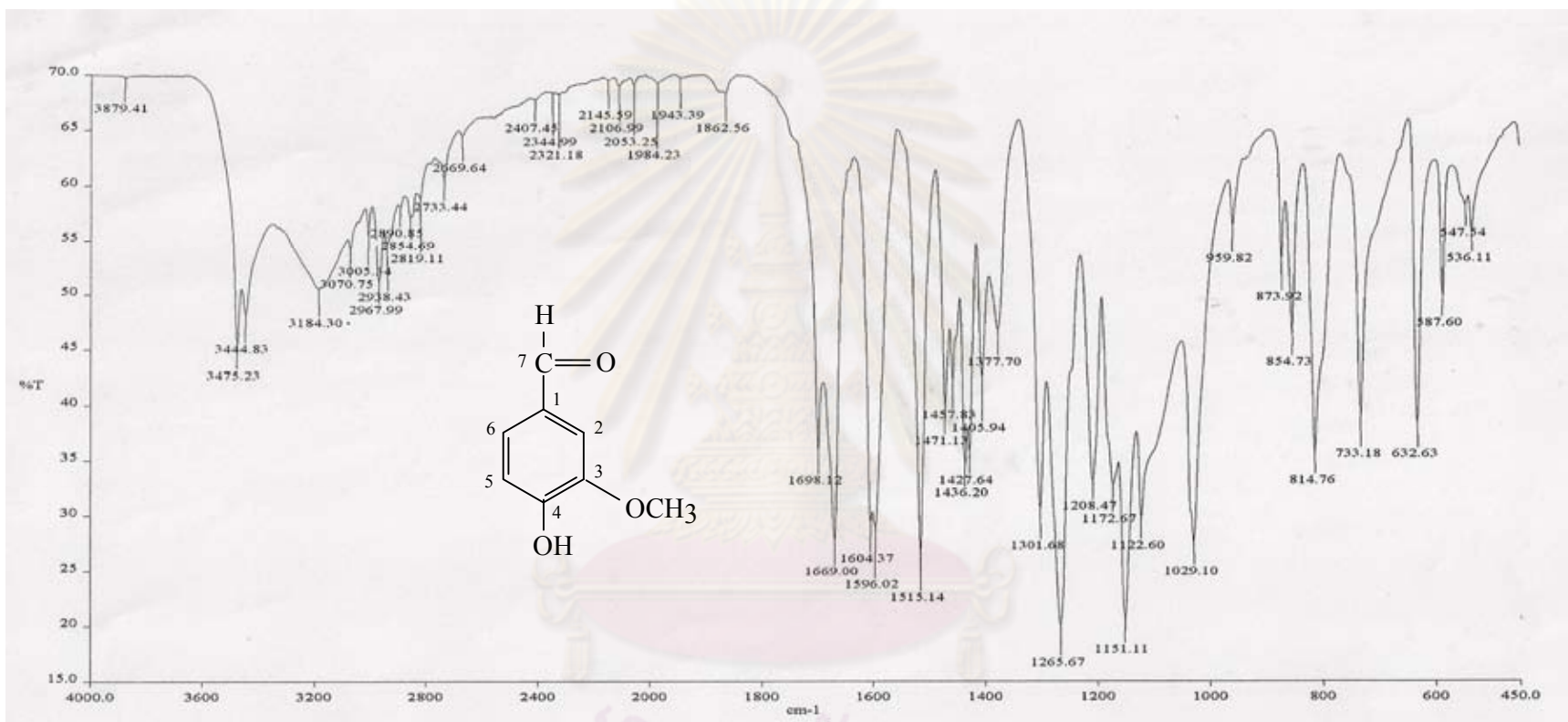
Figure 10. IR Spectrum of compound MS-2 (KBr)
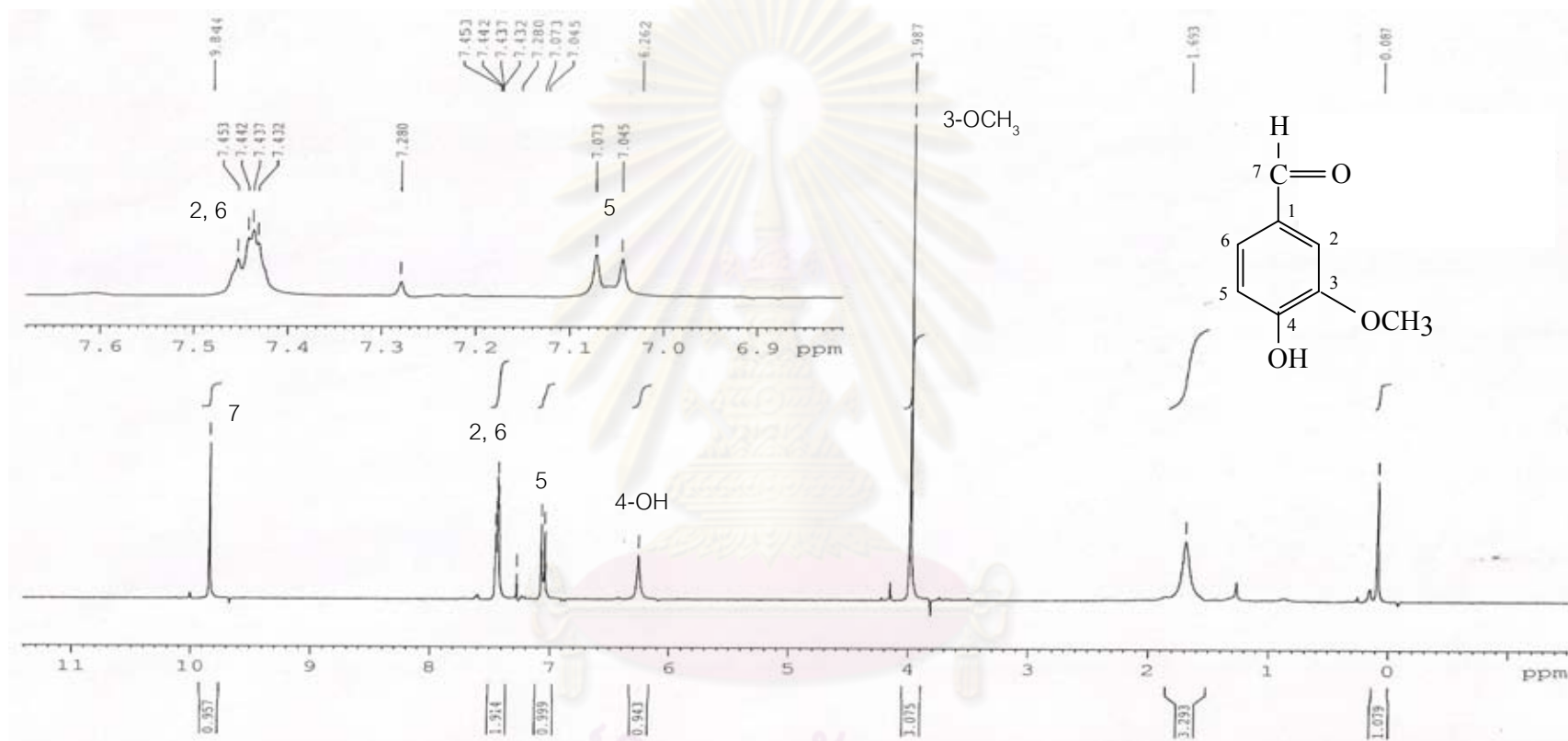
Figure 11. ^{1}H NMR (300 MHz) Spectrum of compound MS-2 (in CDCl$_3$)
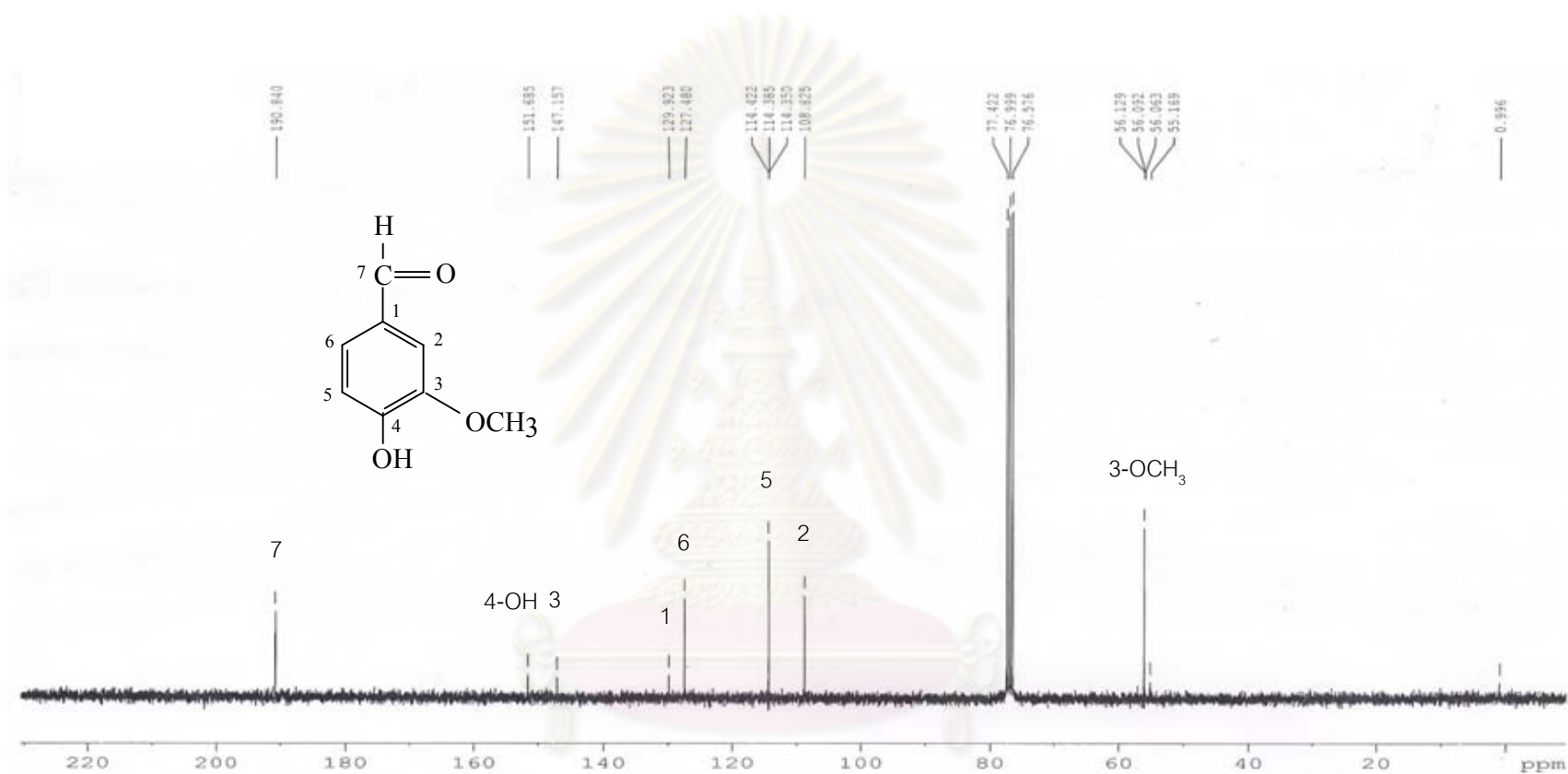
Figure 12. ^{13}C NMR (75 MHz) Spectrum of compound MS-2 (in CDCl$_3$)
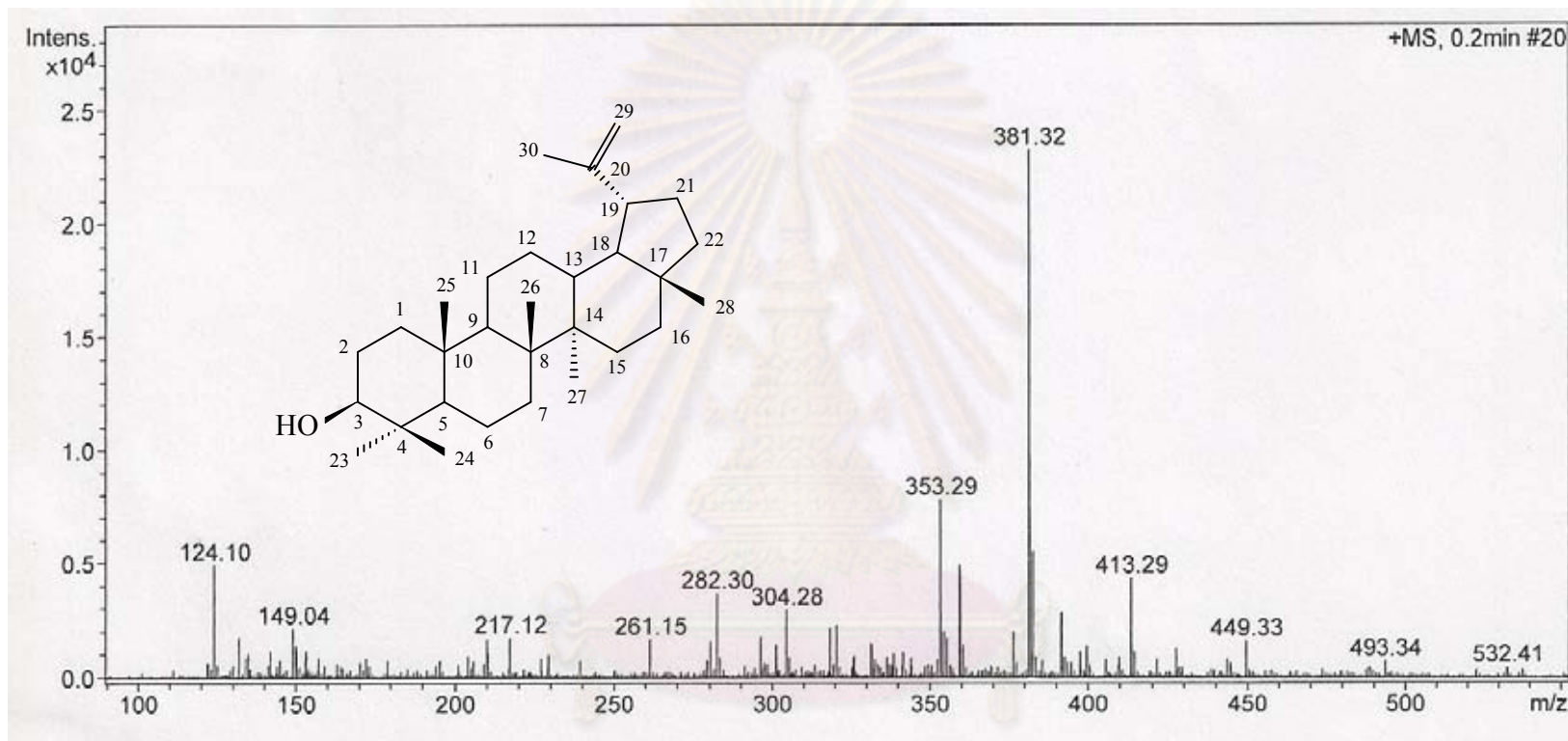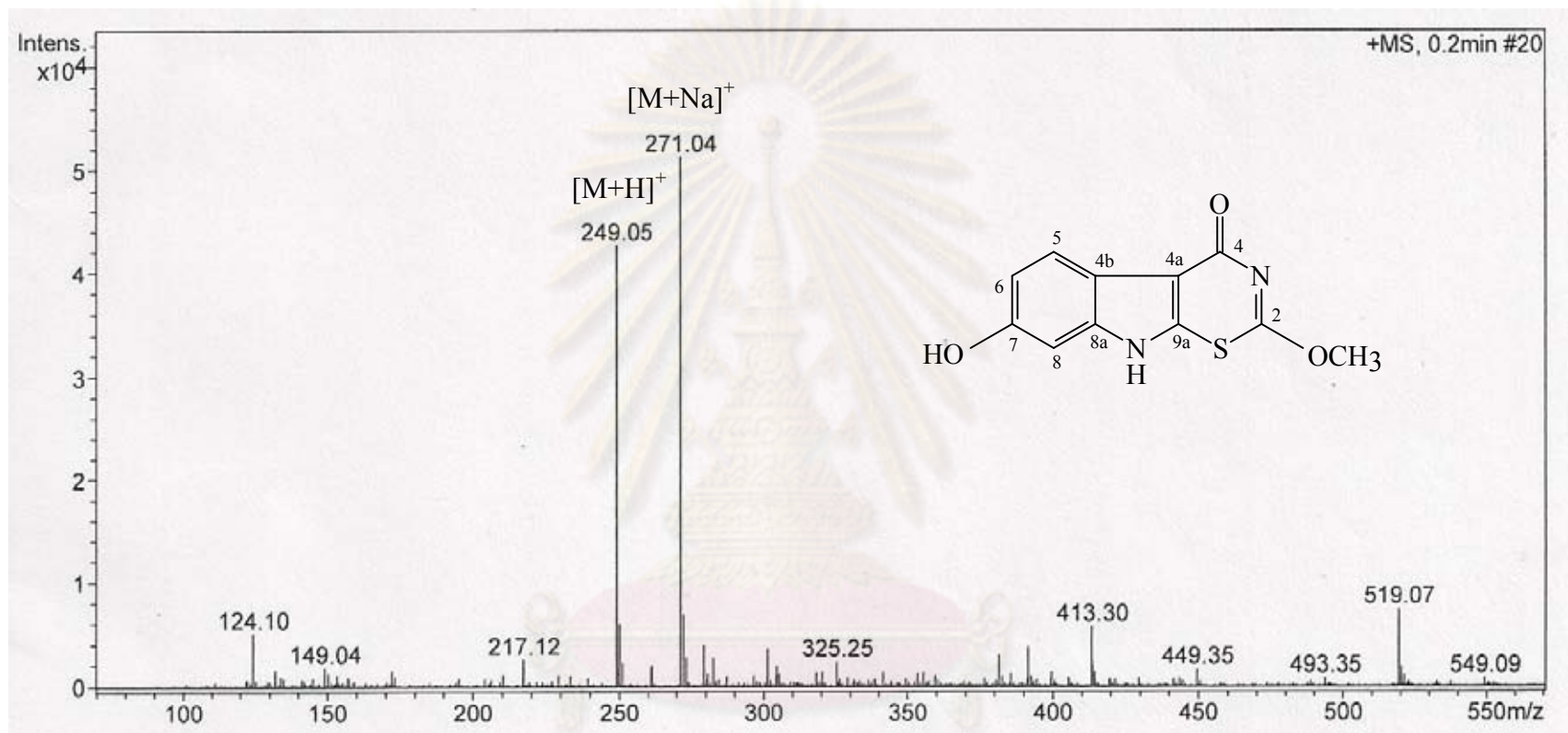
Figure 13. ESI Mass spectrum of compound MS-3
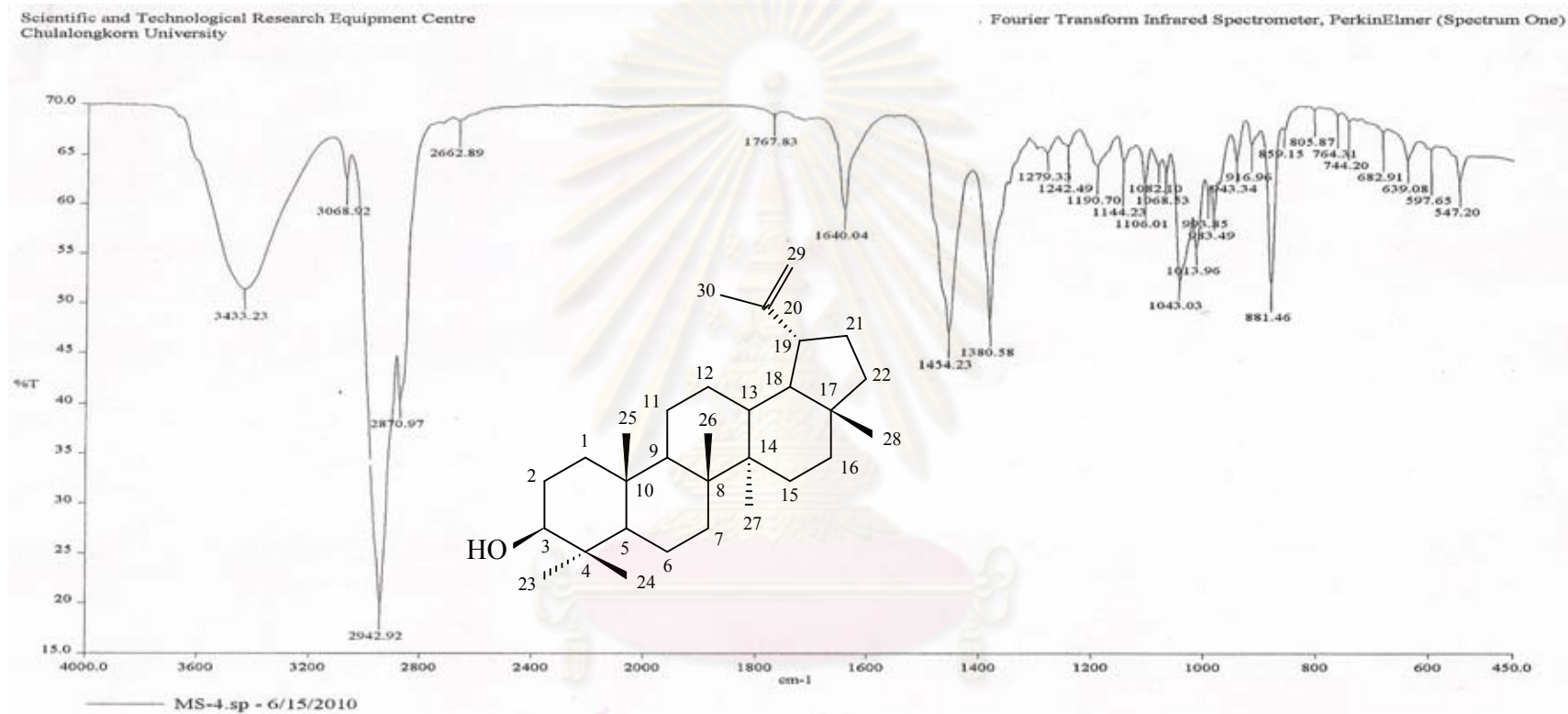
Figure 14. IR Spectrum of compound MS-3 (KBr)
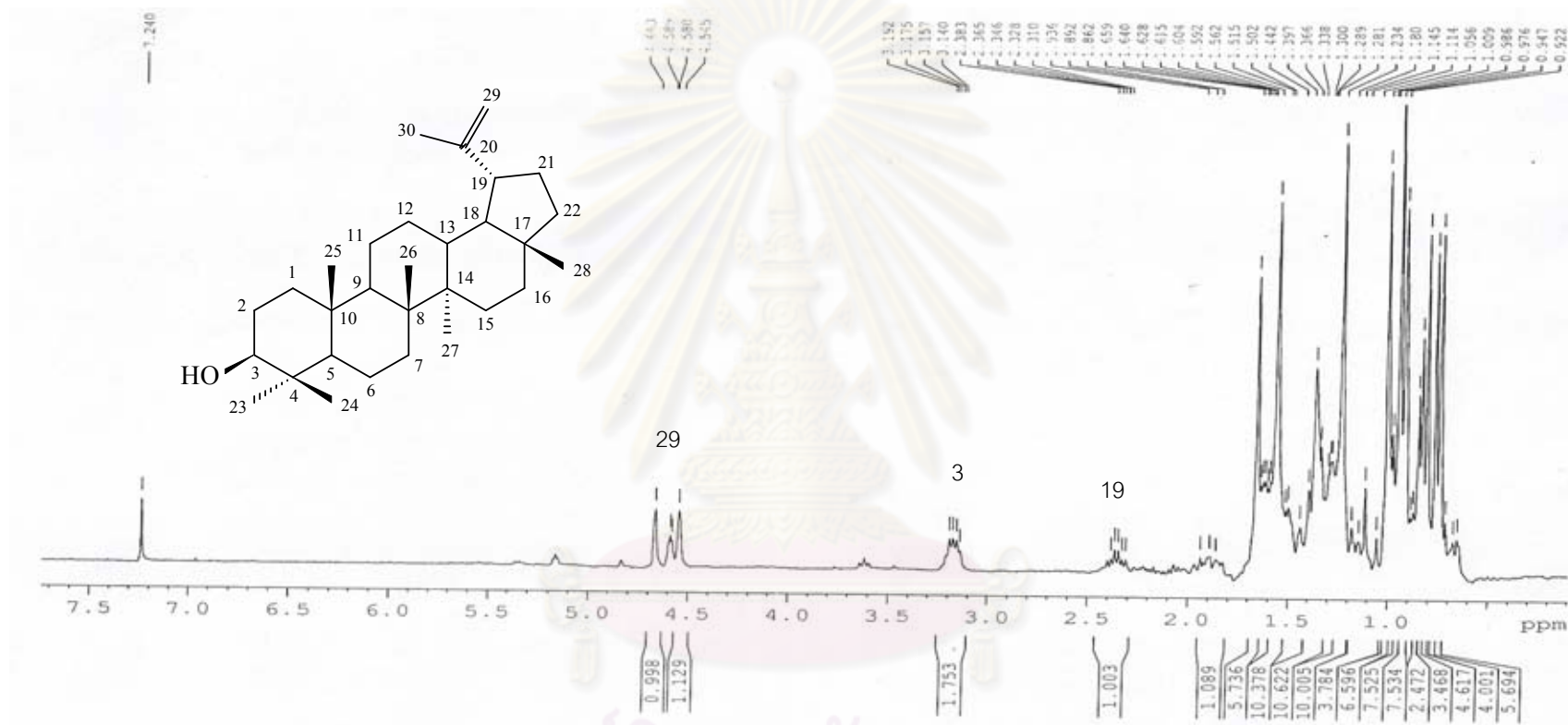
Figure 15a. ¹H NMR (300 MHz) Spectrum of compound MS-3 (in CDCl₃)
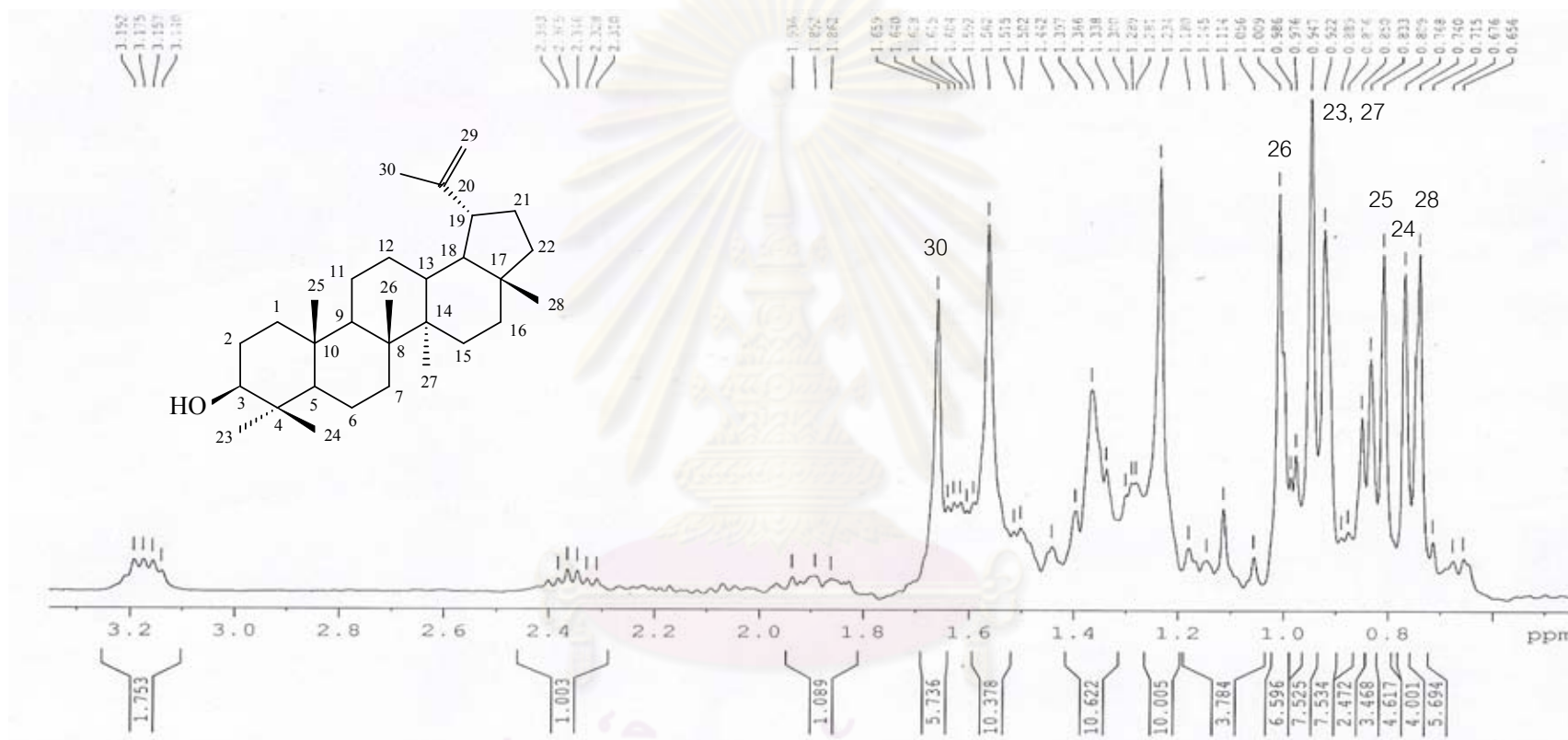
Figure 15b. ^{1}H NMR (300 MHz) Spectrum of compound MS-3 (in CDCl$_3$) (expansion between δ 0.6-3.2 ppm)
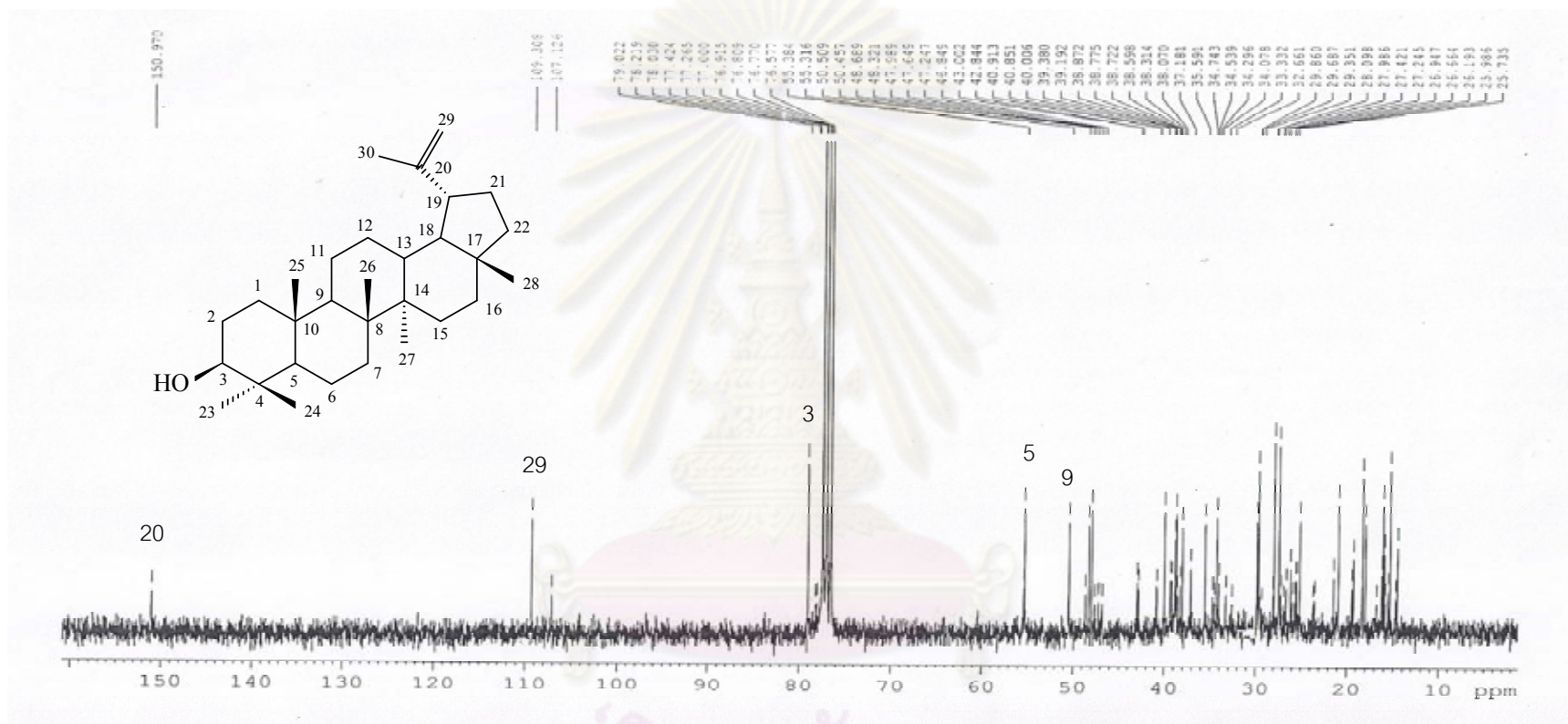
Figure 16a. ^{13}C NMR (75 MHz) Spectrum of compound MS-3 (in CDCl$_3$)
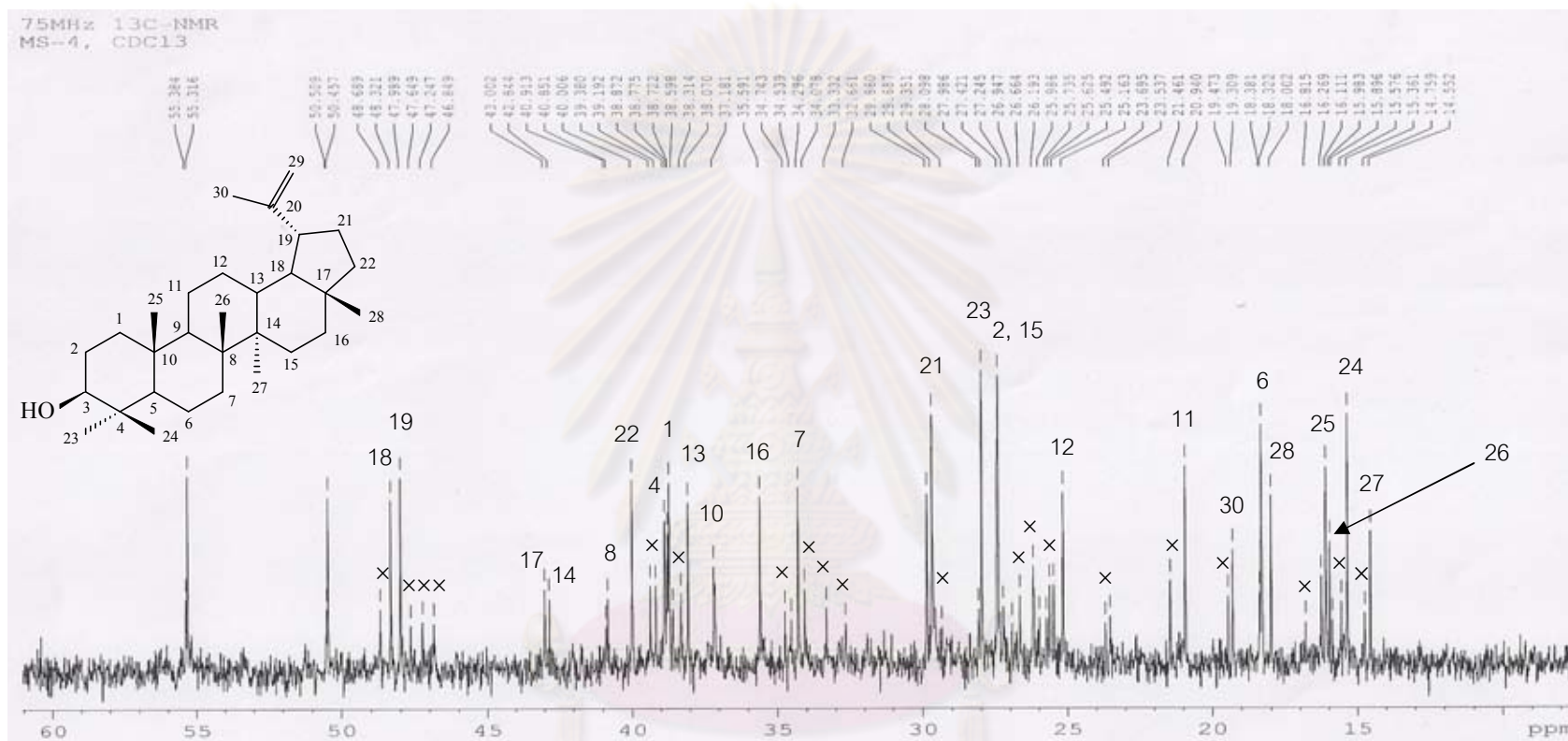
Figure 16b. ^{13}C NMR (75 MHz) Spectrum of compound MS-3 (in CDCl$_3$) (expansion between δ 0-60 ppm)

Figure 17. UV Spectrum of compound MS-4 (in MeOH)
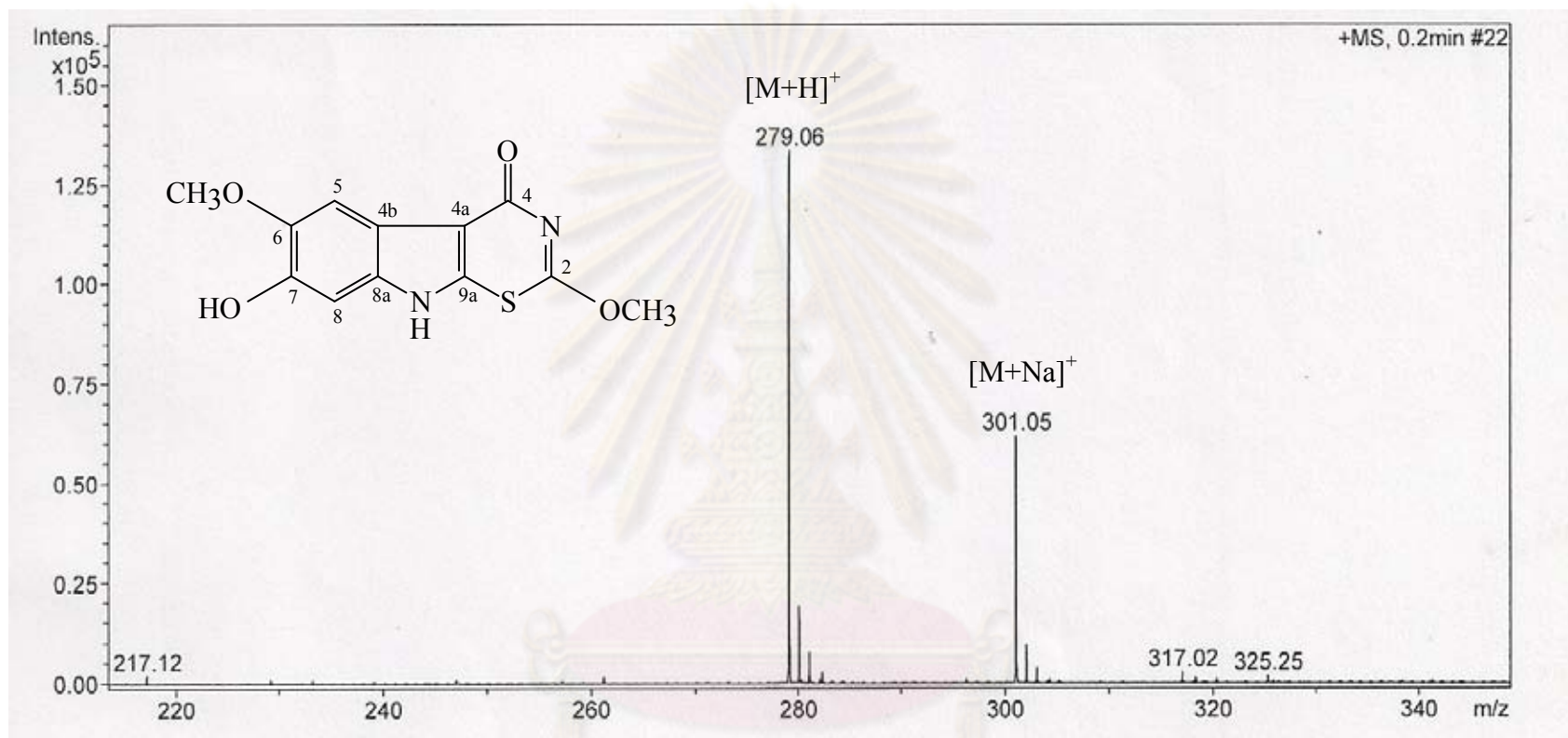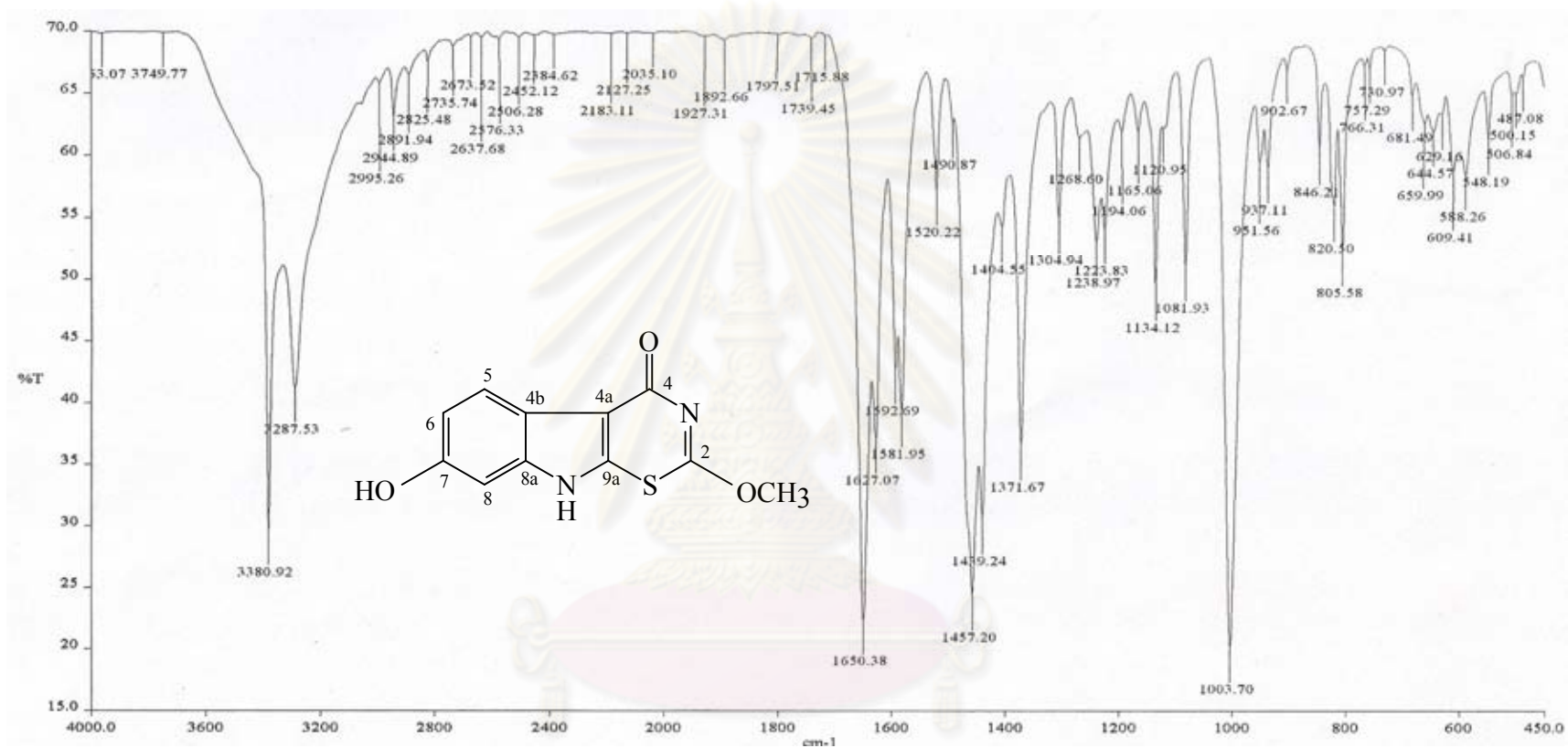
Figure 18. ESI Mass spectrum of compound MS-4
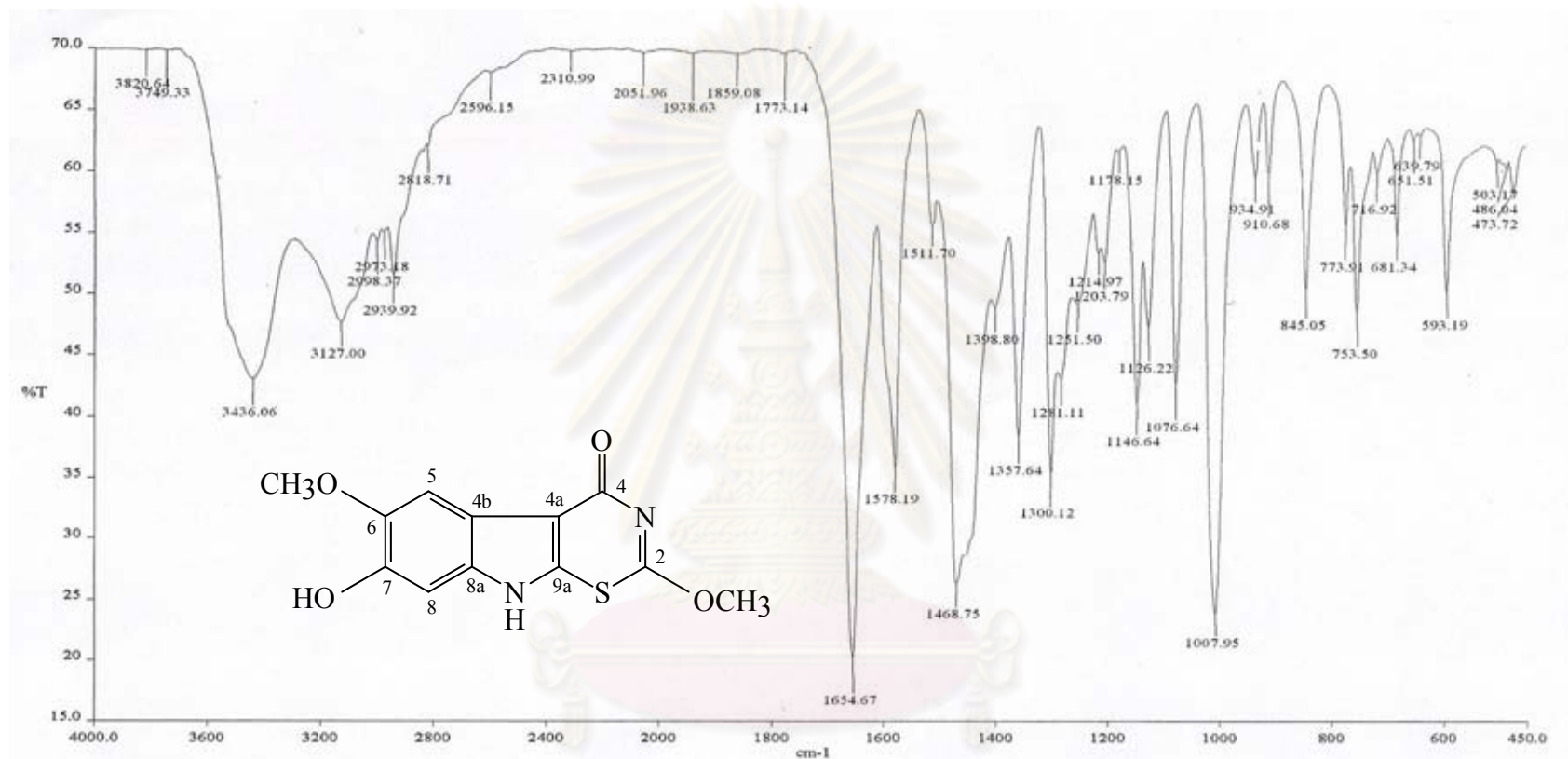
Figure 19. IR Spectrum of compound MS-4
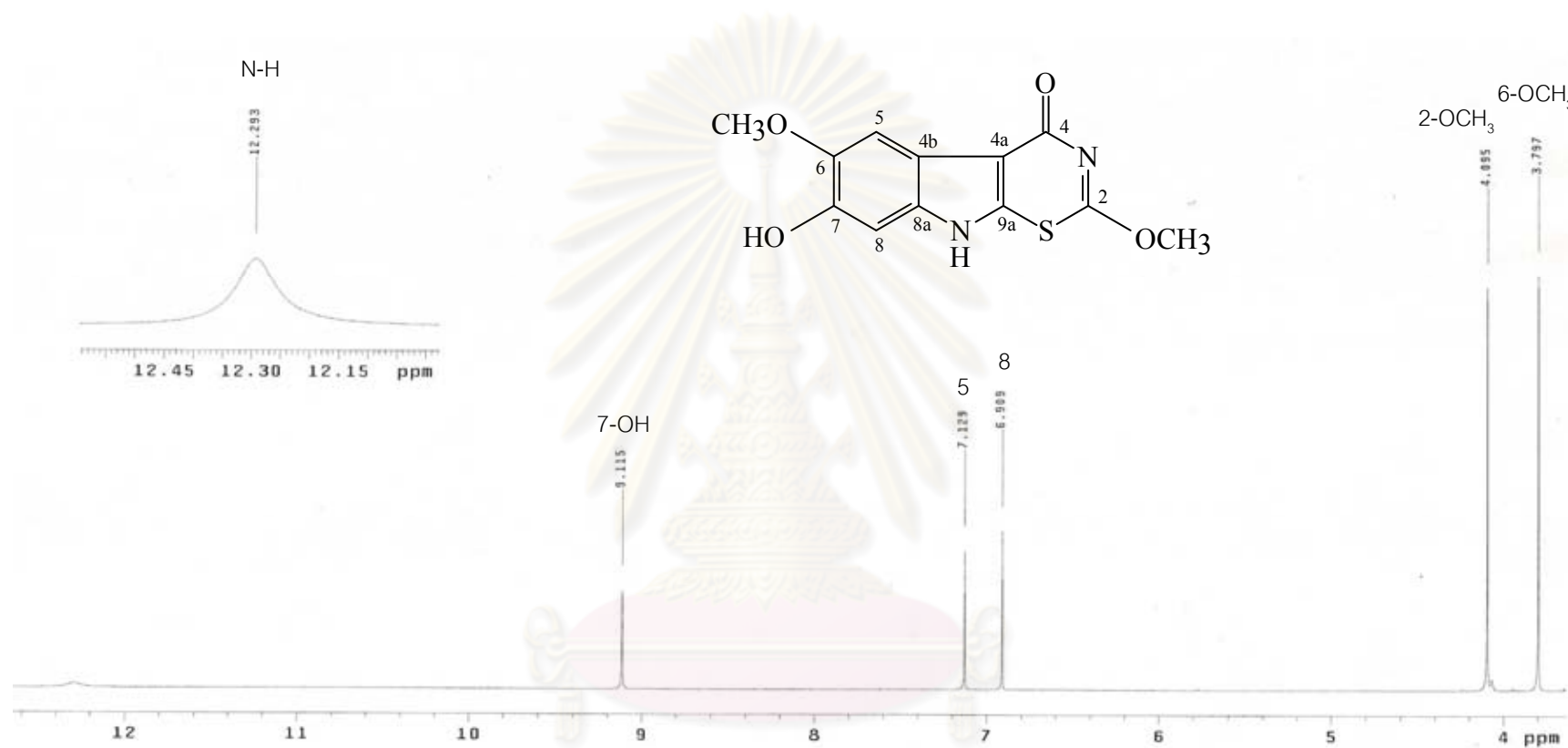
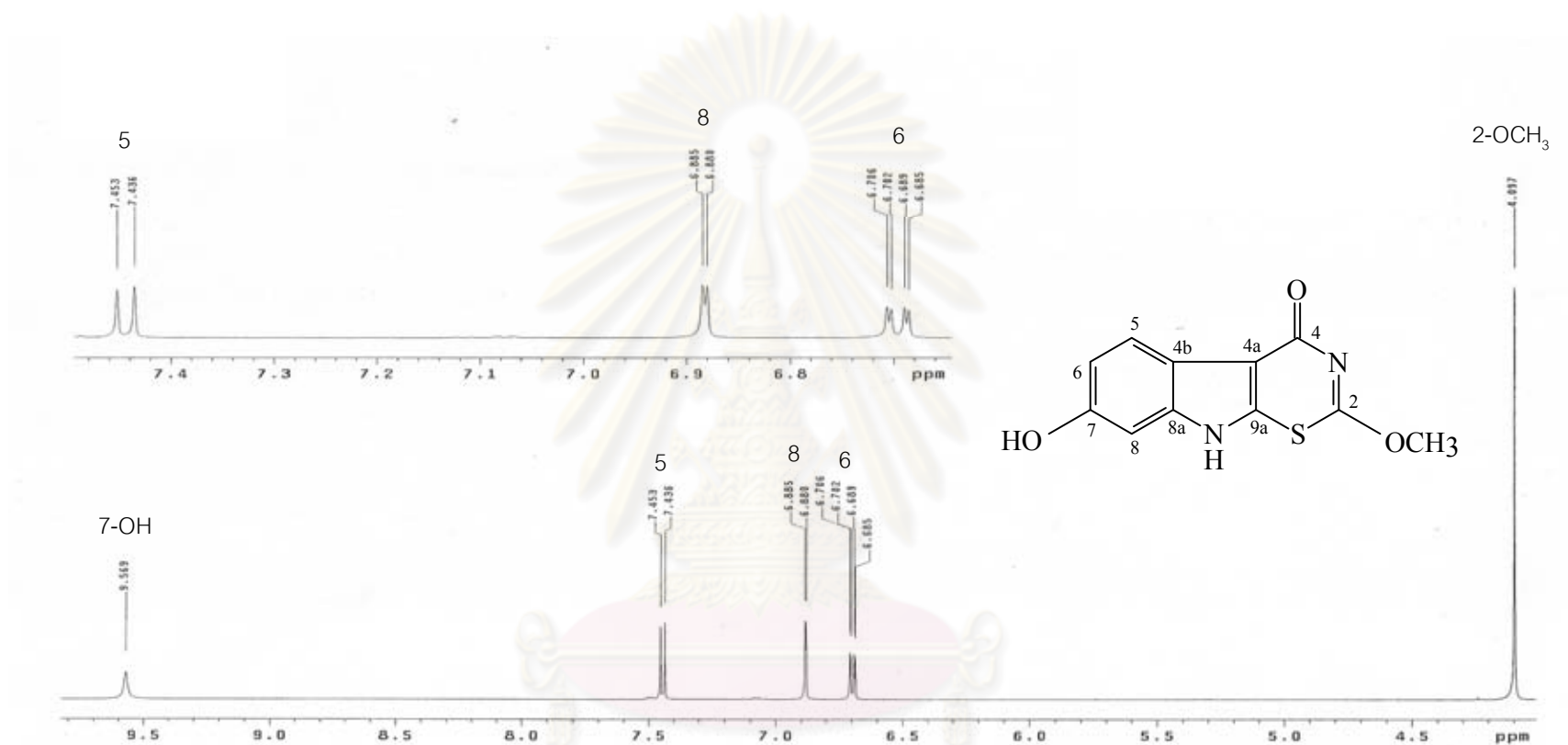Figure 20. ^{1}H NMR (500 MHz) Spectrum of compound MS-4 (in DMSO-d$_6$)
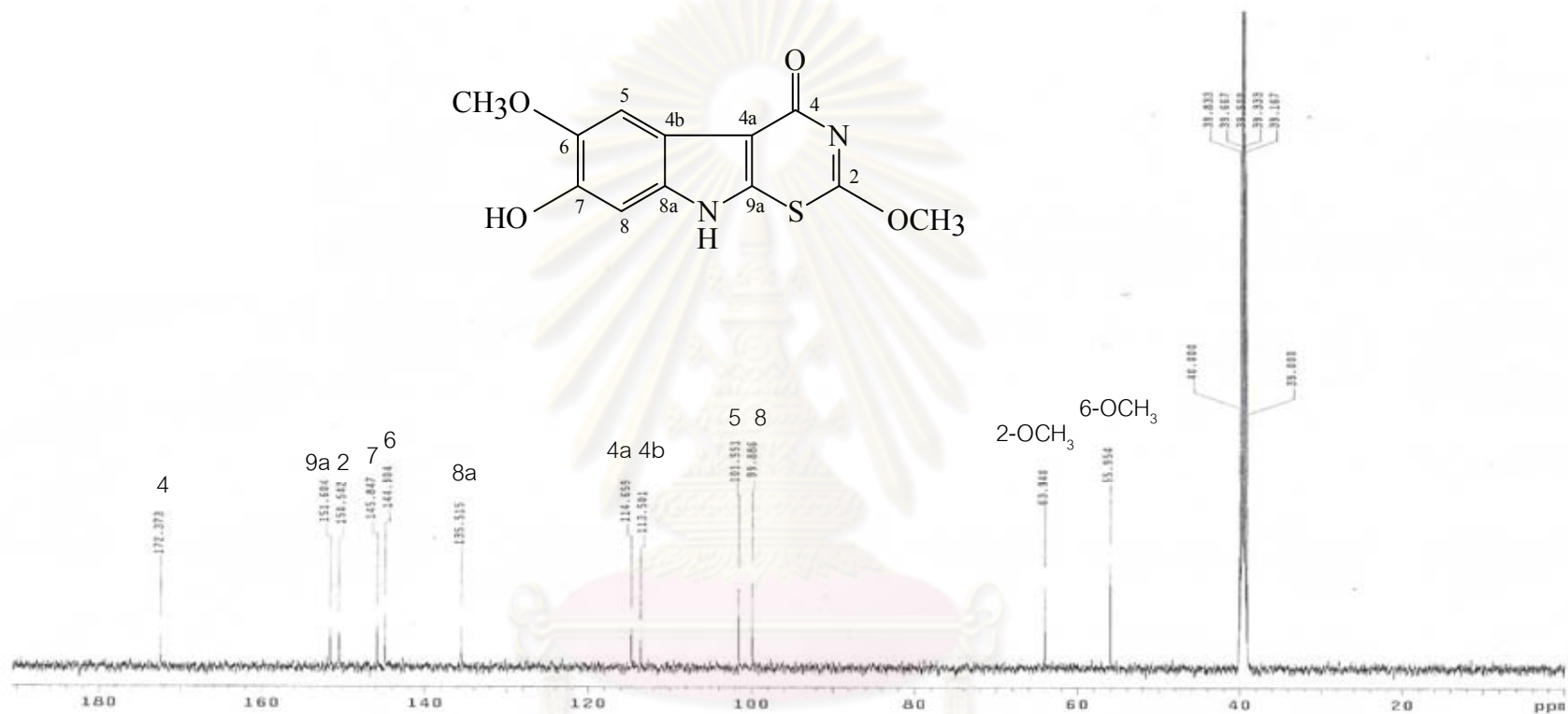
Figure 21. ^{13}C NMR (125 MHz) Spectrum of compound MS-4 (in DMSO-d$_6$)
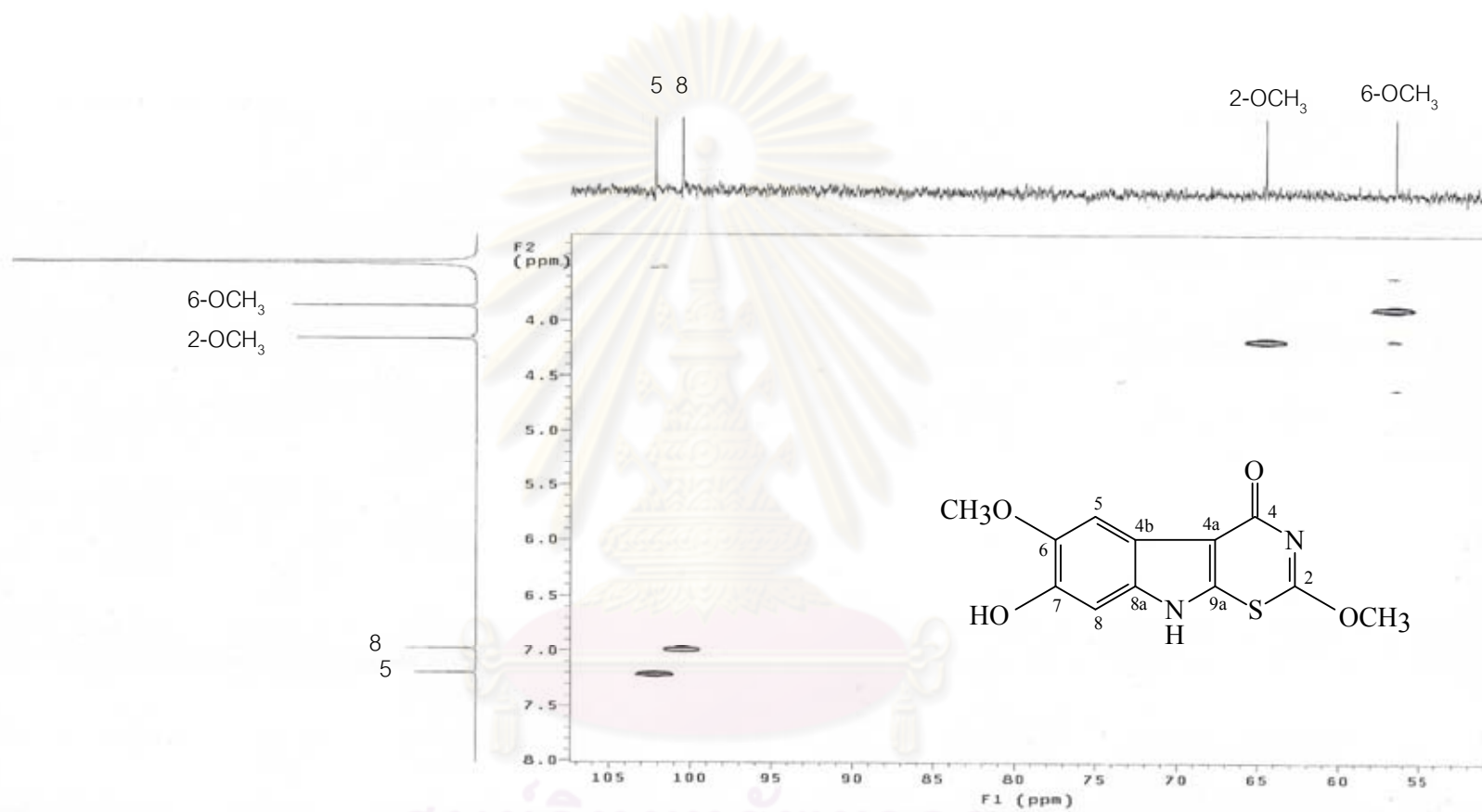
Figure 22. HSQC Spectrum of compound MS-4 (in DMSO-d_6)
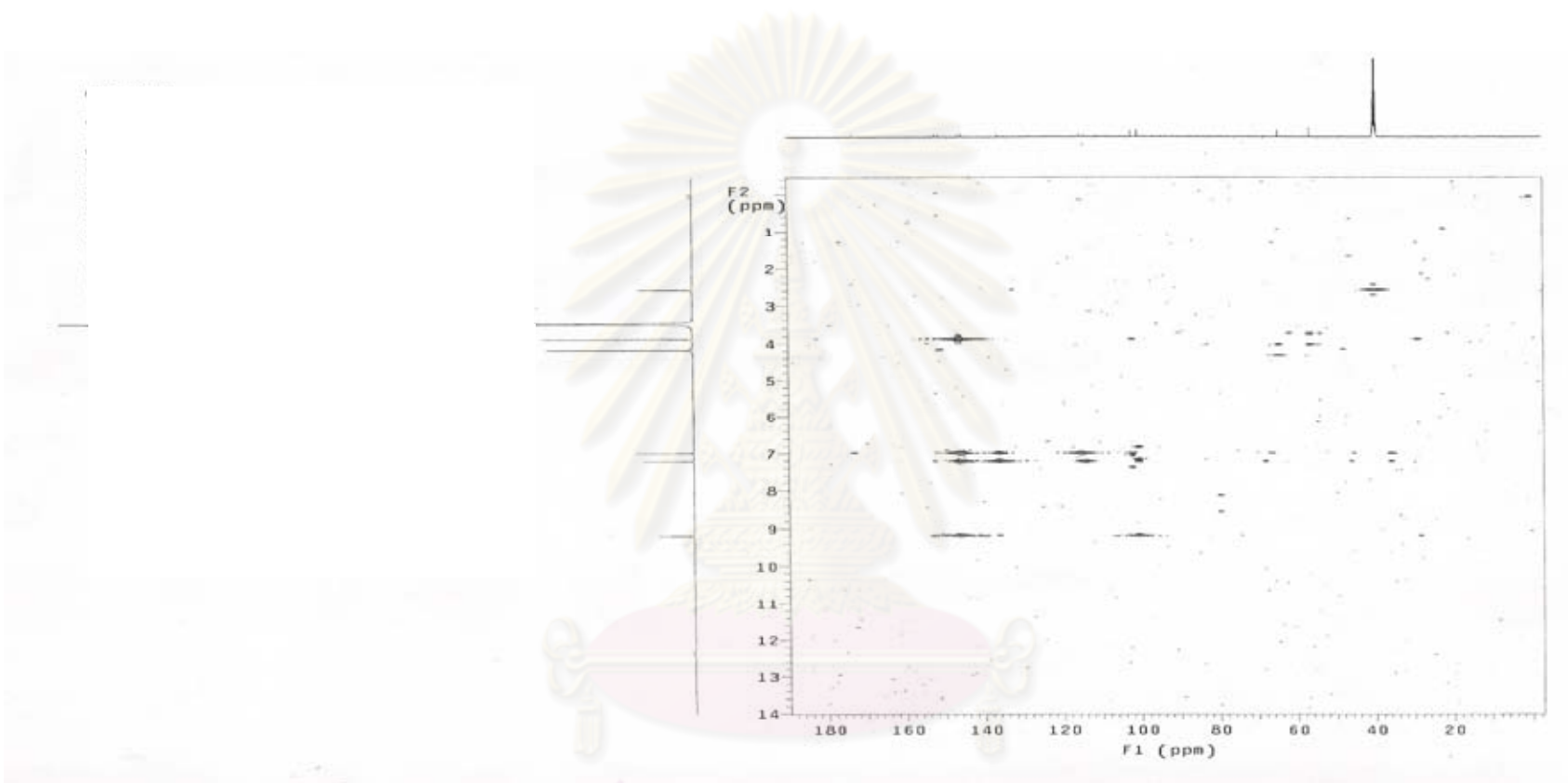
Figure 23a. HMBC Spectrum of compound MS-4
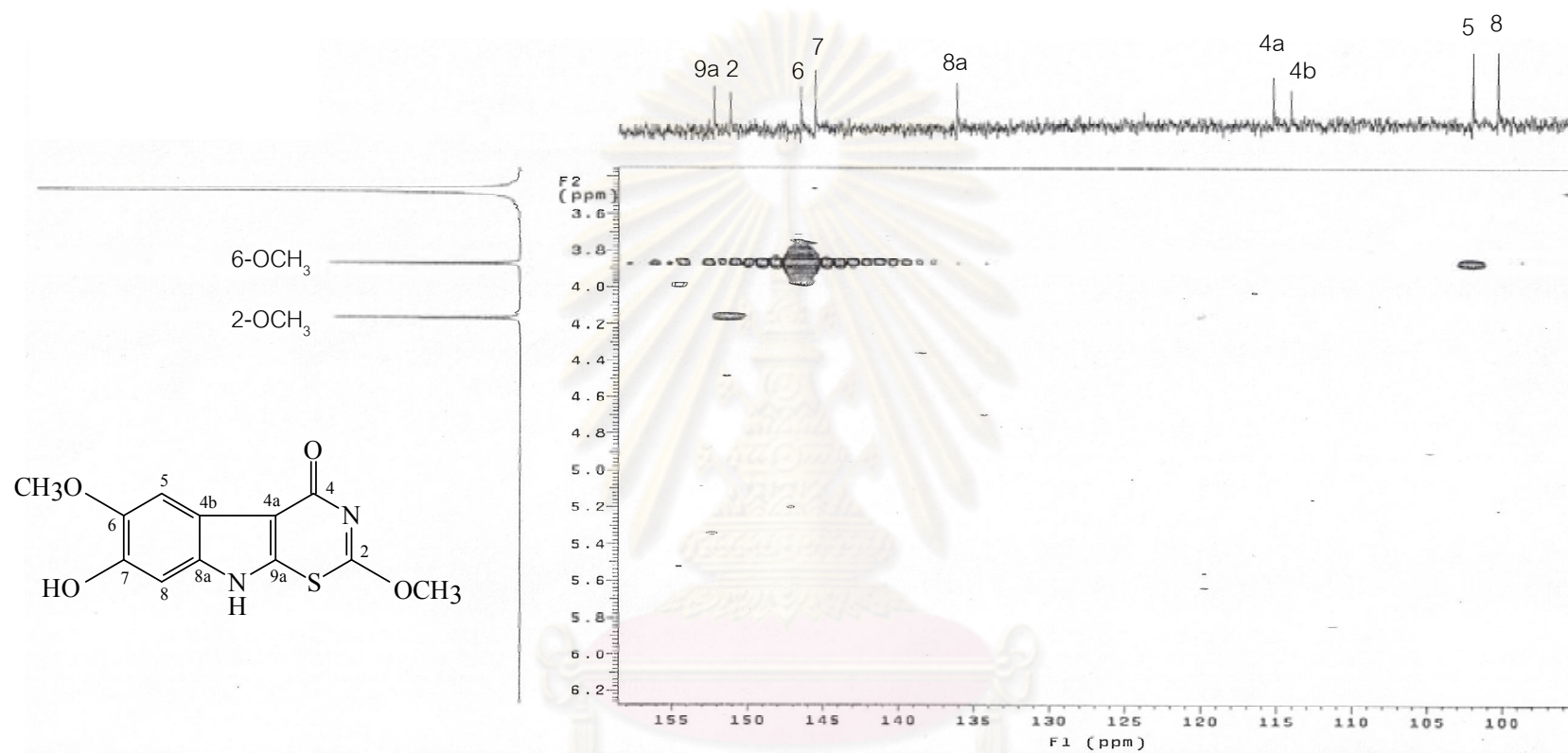
Figure 23b. HMBC Spectrum of compound MS-4 (expansion between δ_H 3.4-6.2 ppm, δ_C 95-160 ppm)
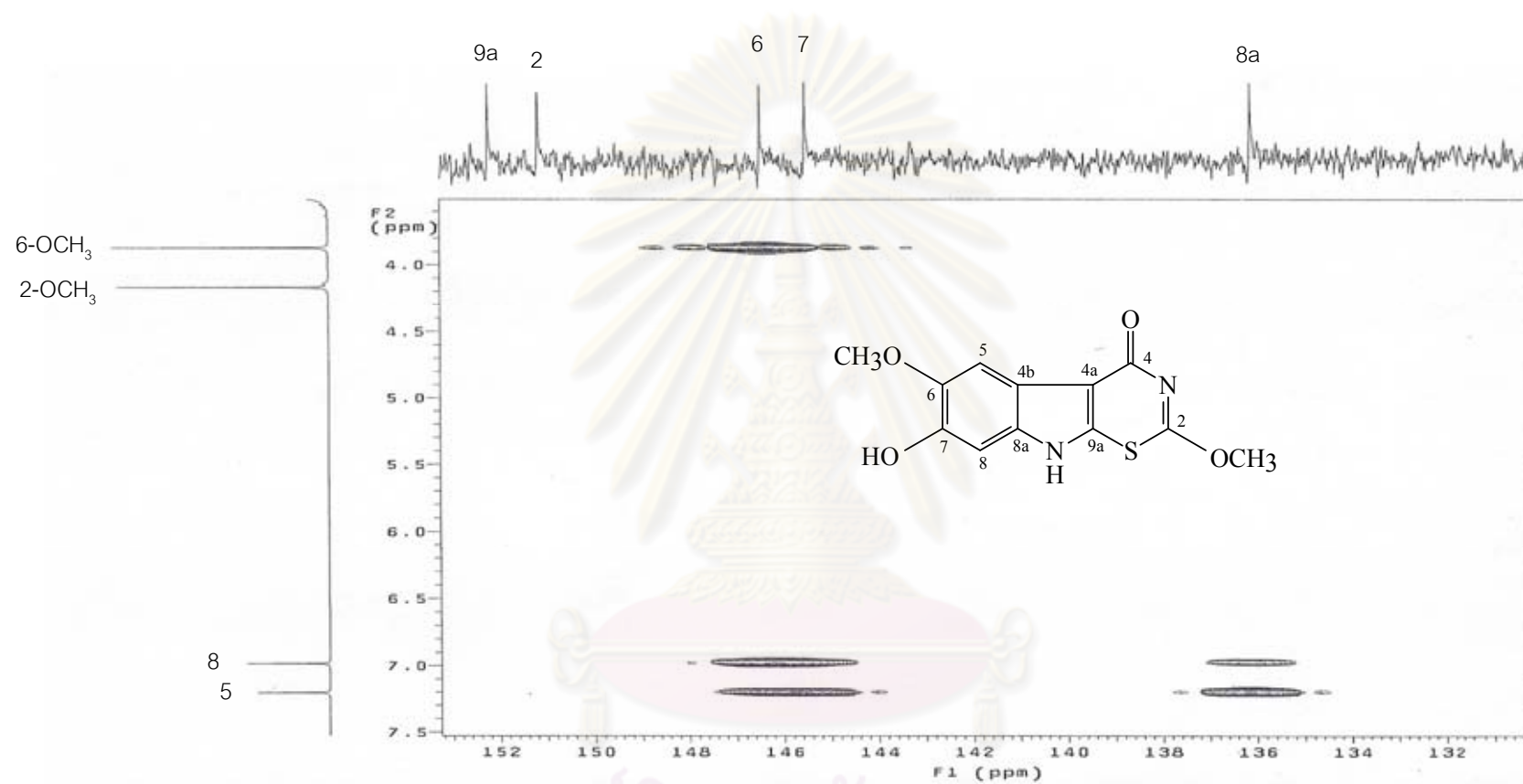
Figure 23c. HMBC Spectrum of compound MS-4 (expansion between δ_H 3.5-7.5 ppm, δ_C 130-153 ppm)
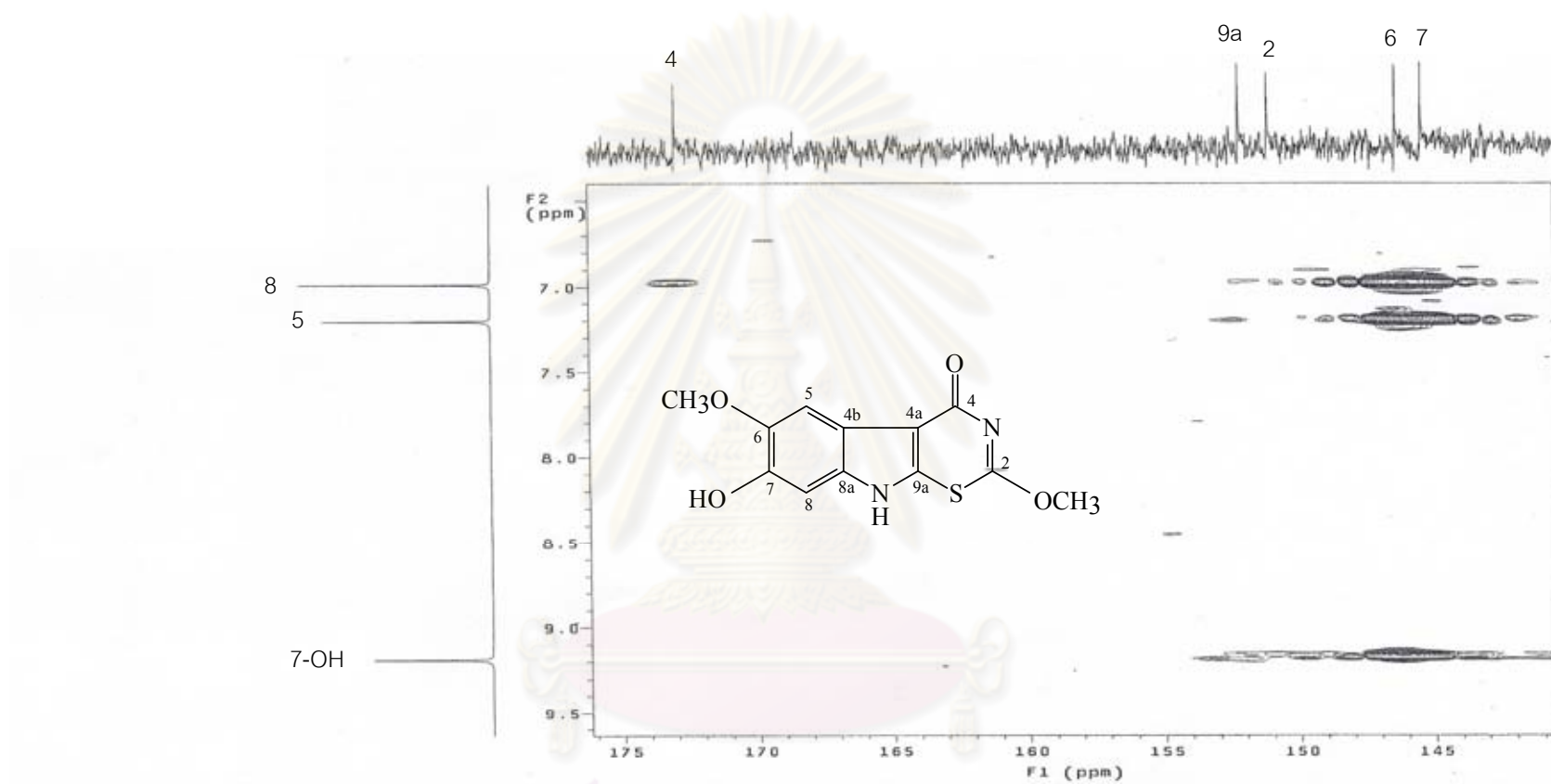
Figure 23d. HMBC Spectrum of compound MS-4 (expansion between δ_H 6.5-9.5 ppm, δ_C 140-175 ppm)
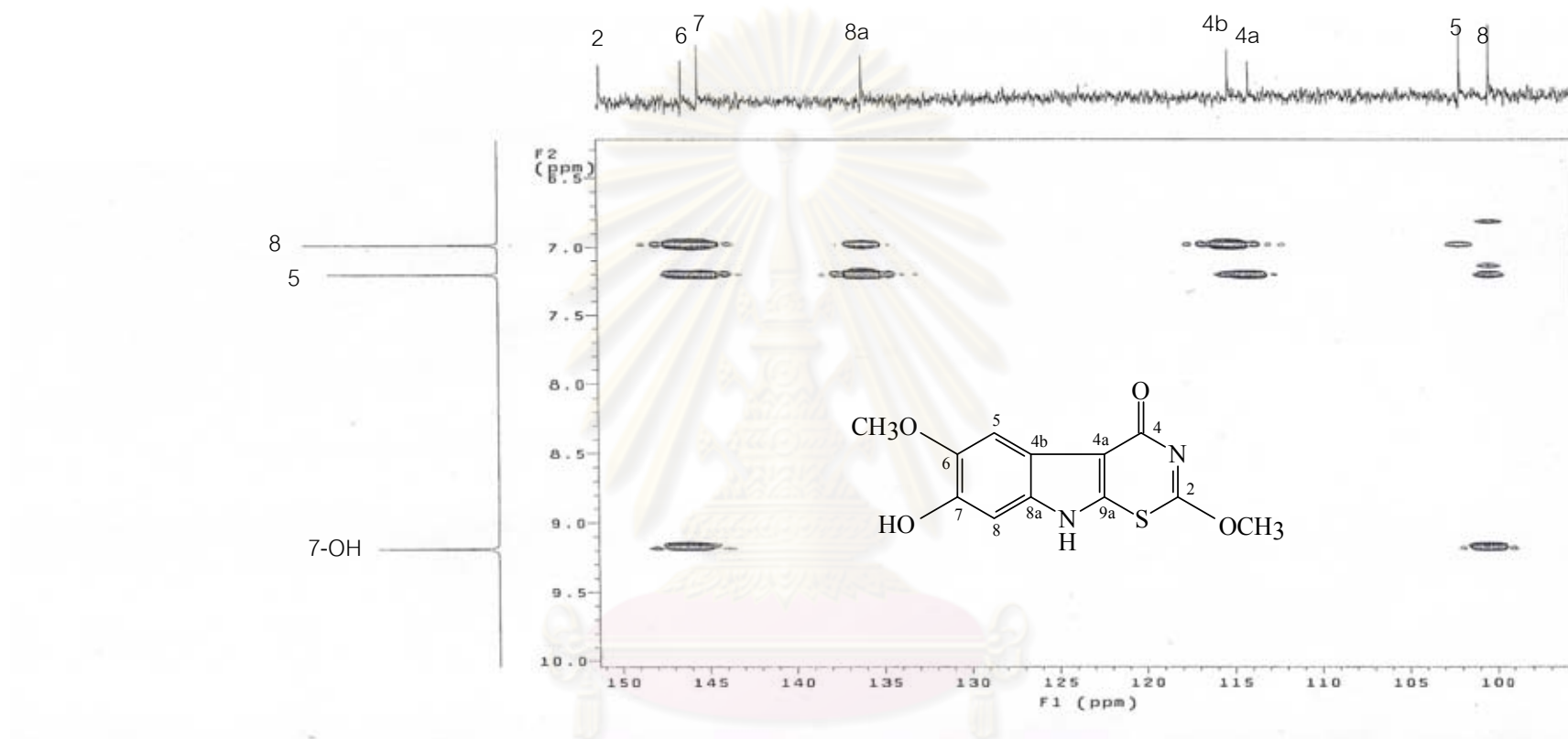
Figure 23e. HMBC Spectrum of compound MS-4 (expansion between δ_H 6.5-10.0 ppm, δ_C 95-150 ppm)
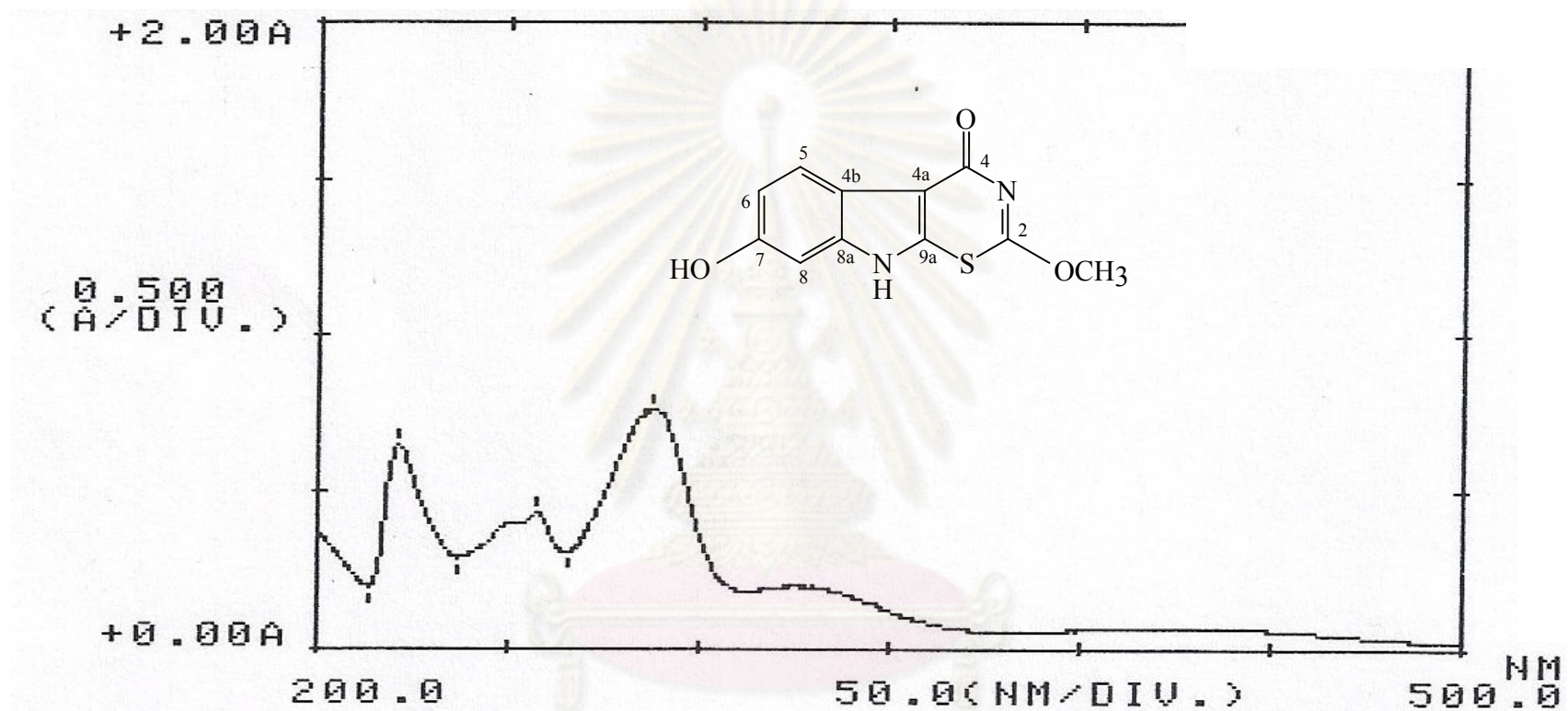
Figure 24. UV Spectrum of compound MS-5 (in MeOH)

Figure 25. ESI Mass spectrum of compound MS-5

Figure 26. IR Spectrum of compound MS-5 (KBr)

Figure 27. ^{1}H NMR (500 MHz) Spectrum of compound MS-5 (in DMSO-d$_6$)
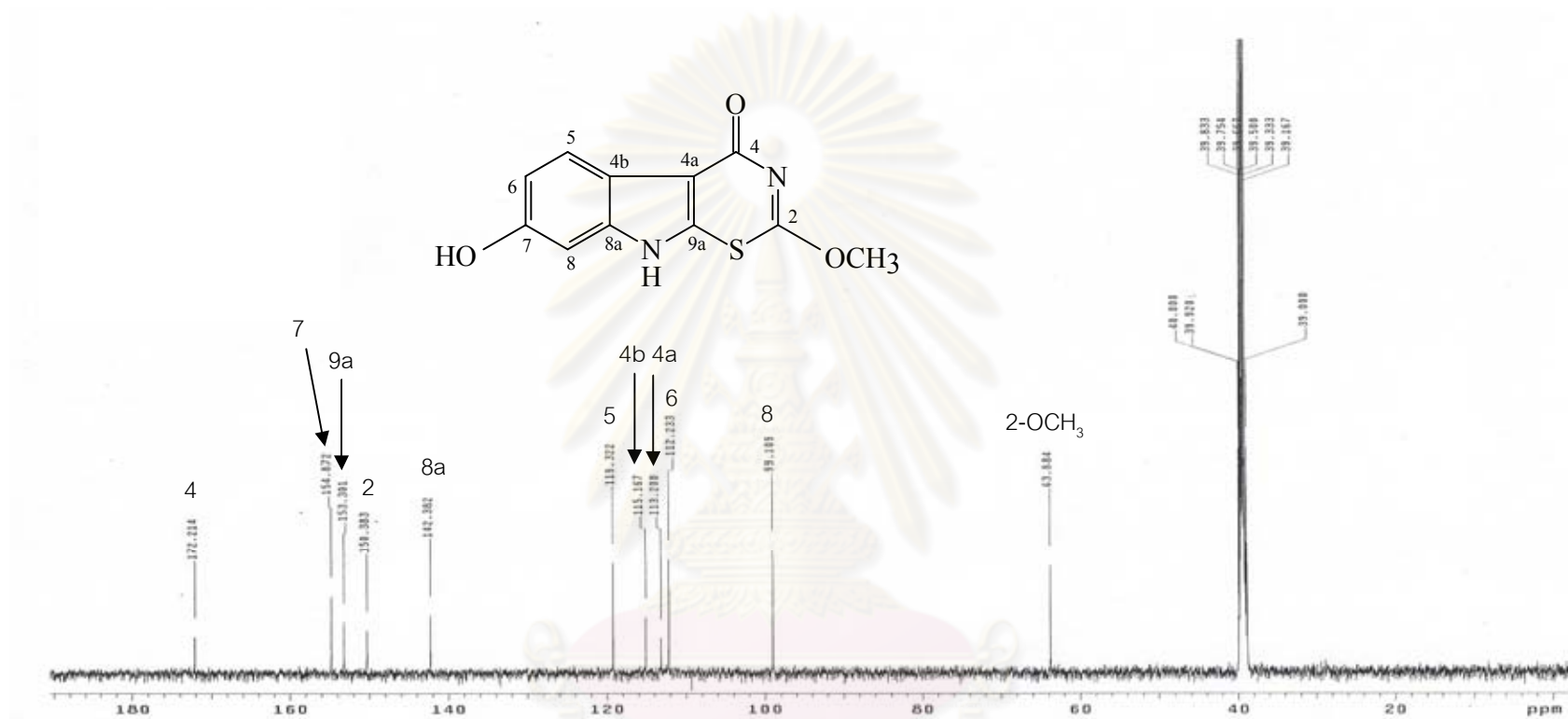
Figure 28. ^{13}C NMR (125 MHz) Spectrum of compound MS-5 (in DMSO-d_6)
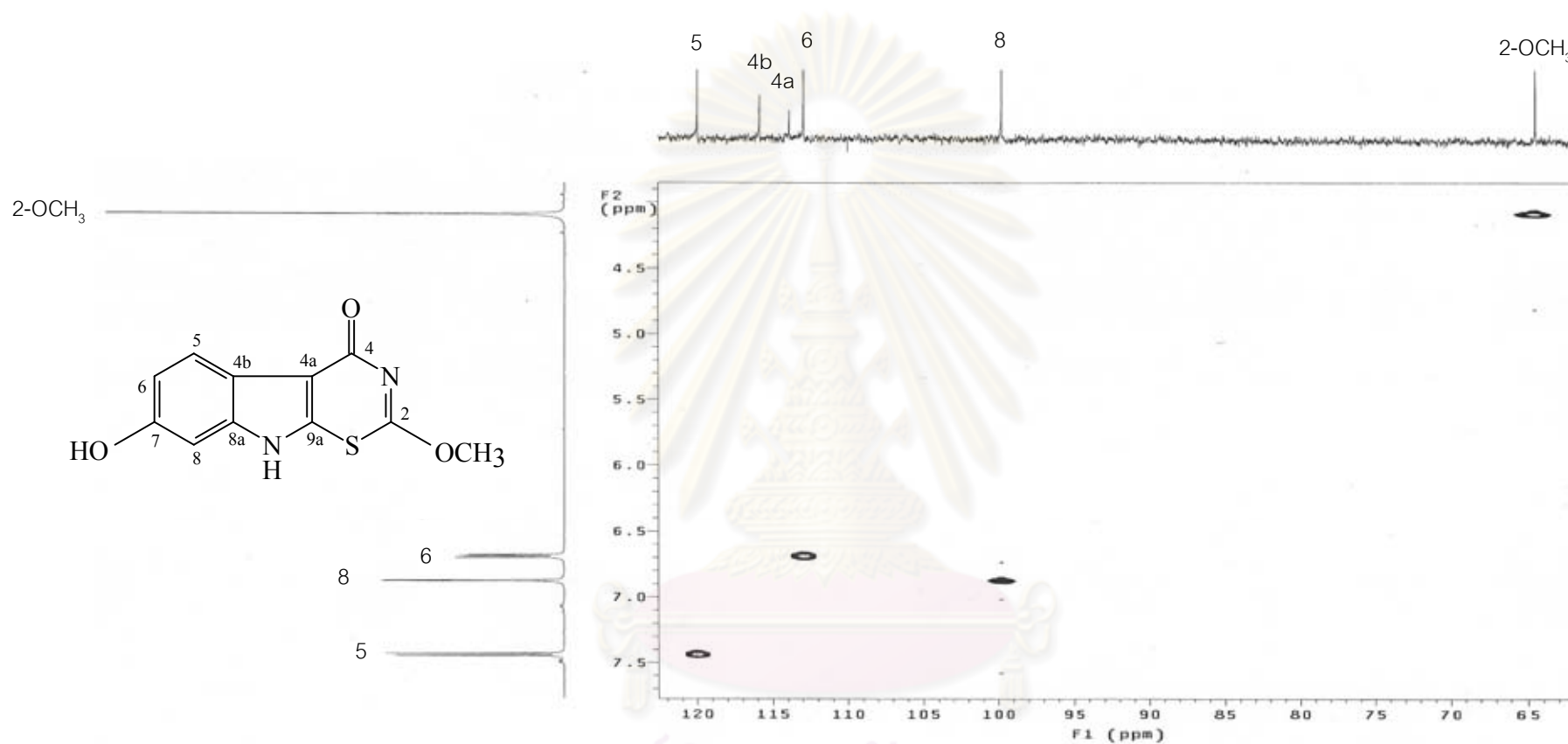
Figure 29. HSQC Spectrum of compound MS-5 (expansion between δ_H 4.0-7.7 ppm, δ_C 62-122 ppm)
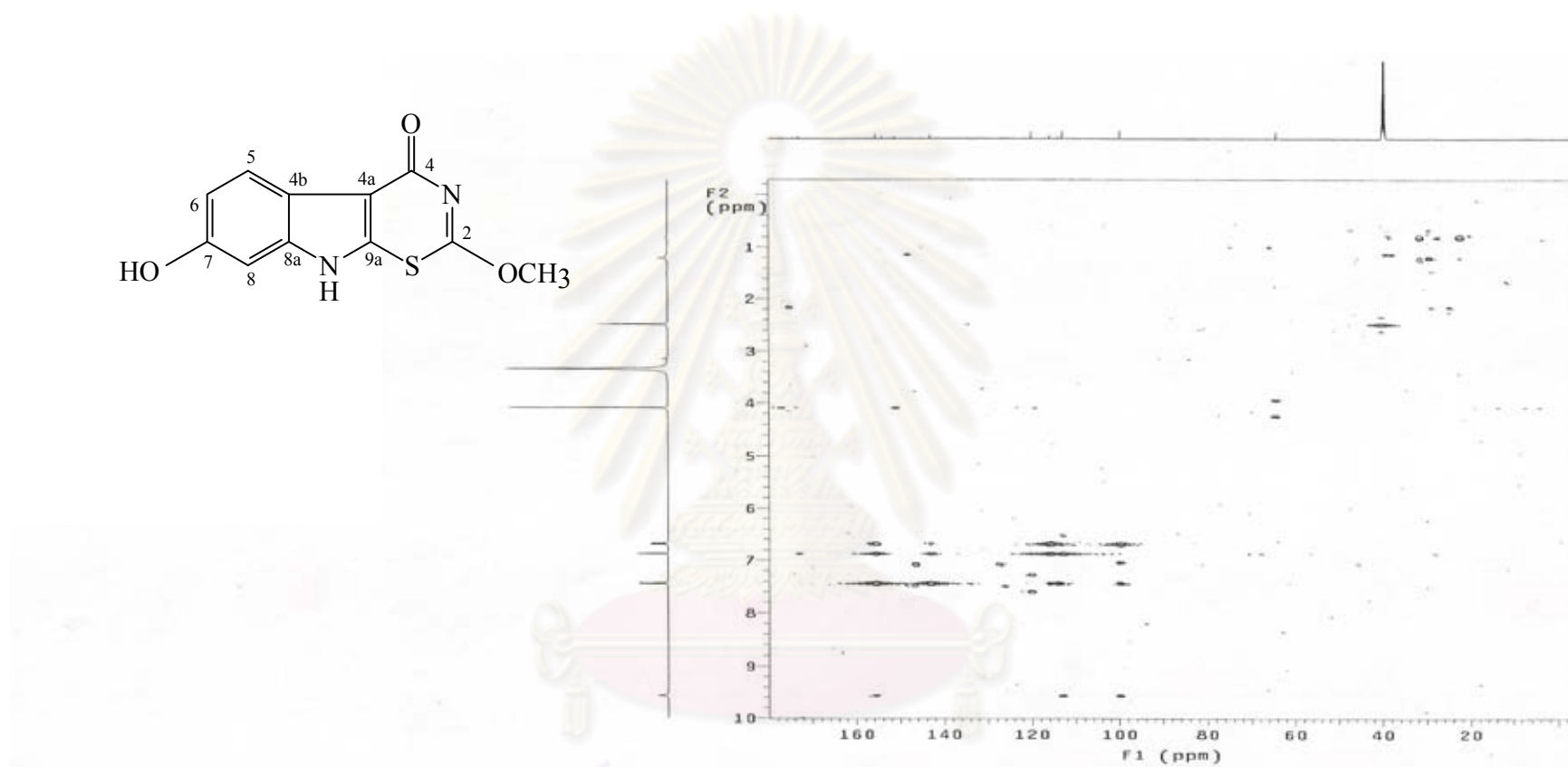
Figure 30a. HMBC Spectrum of compound MS-5 (in DMSO-d_6)
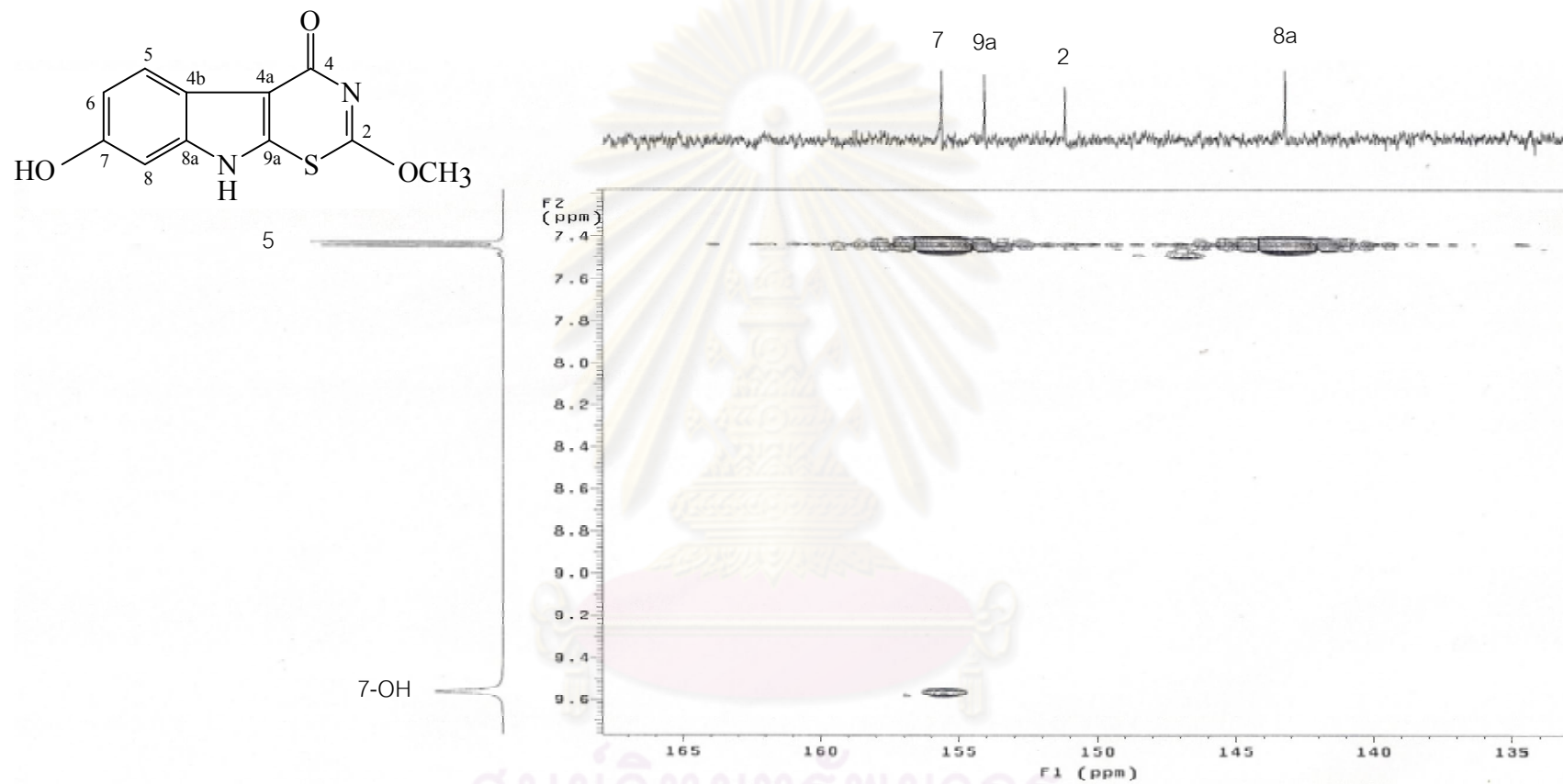
Figure 30b. HMBC Spectrum of compound MS-5 (expansion between δ_H 7.2-9.7 ppm, δ_C 133-168 ppm)
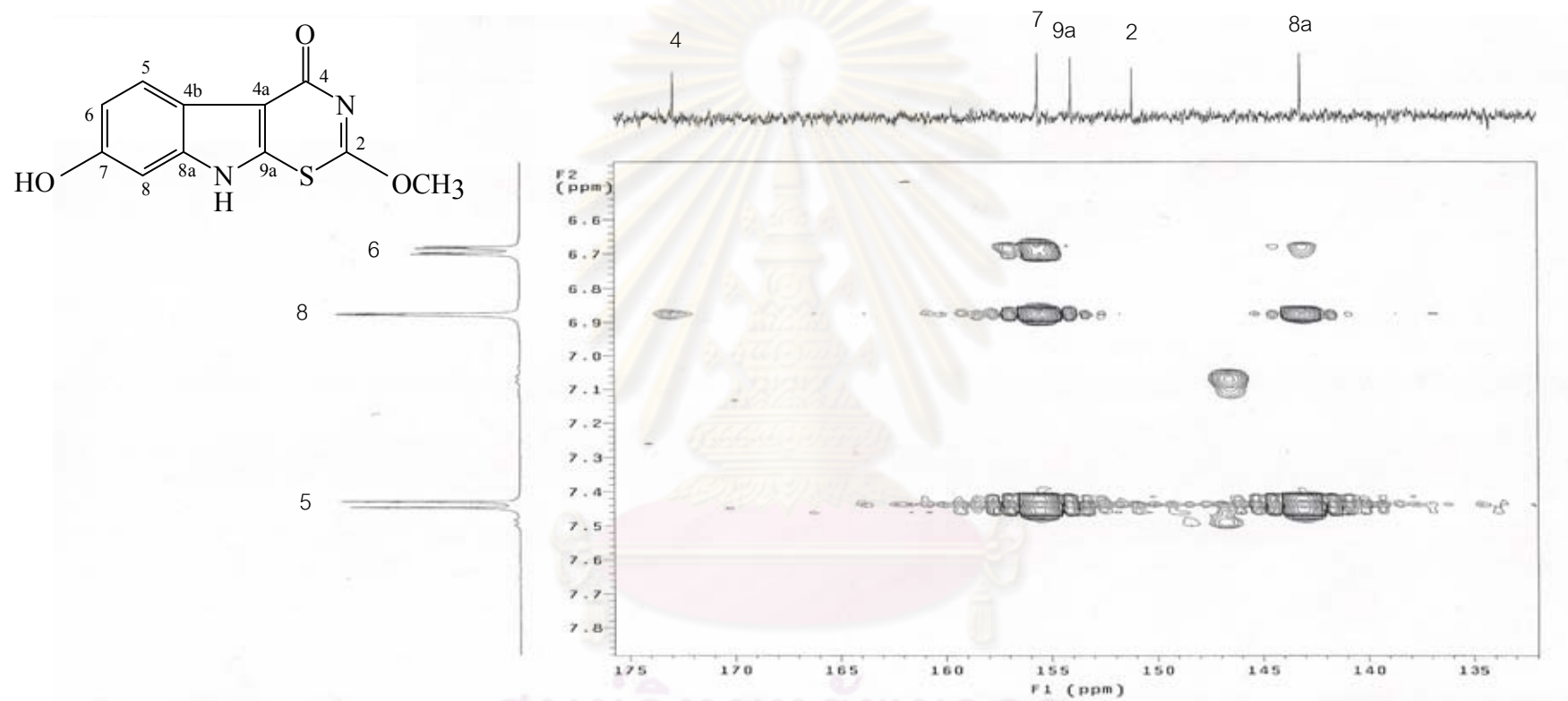
Figure 30c. HMBC Spectrum of compound MS-5 (expansion between δ_H 6.5-7.8 ppm, δ_C 132-175 ppm)
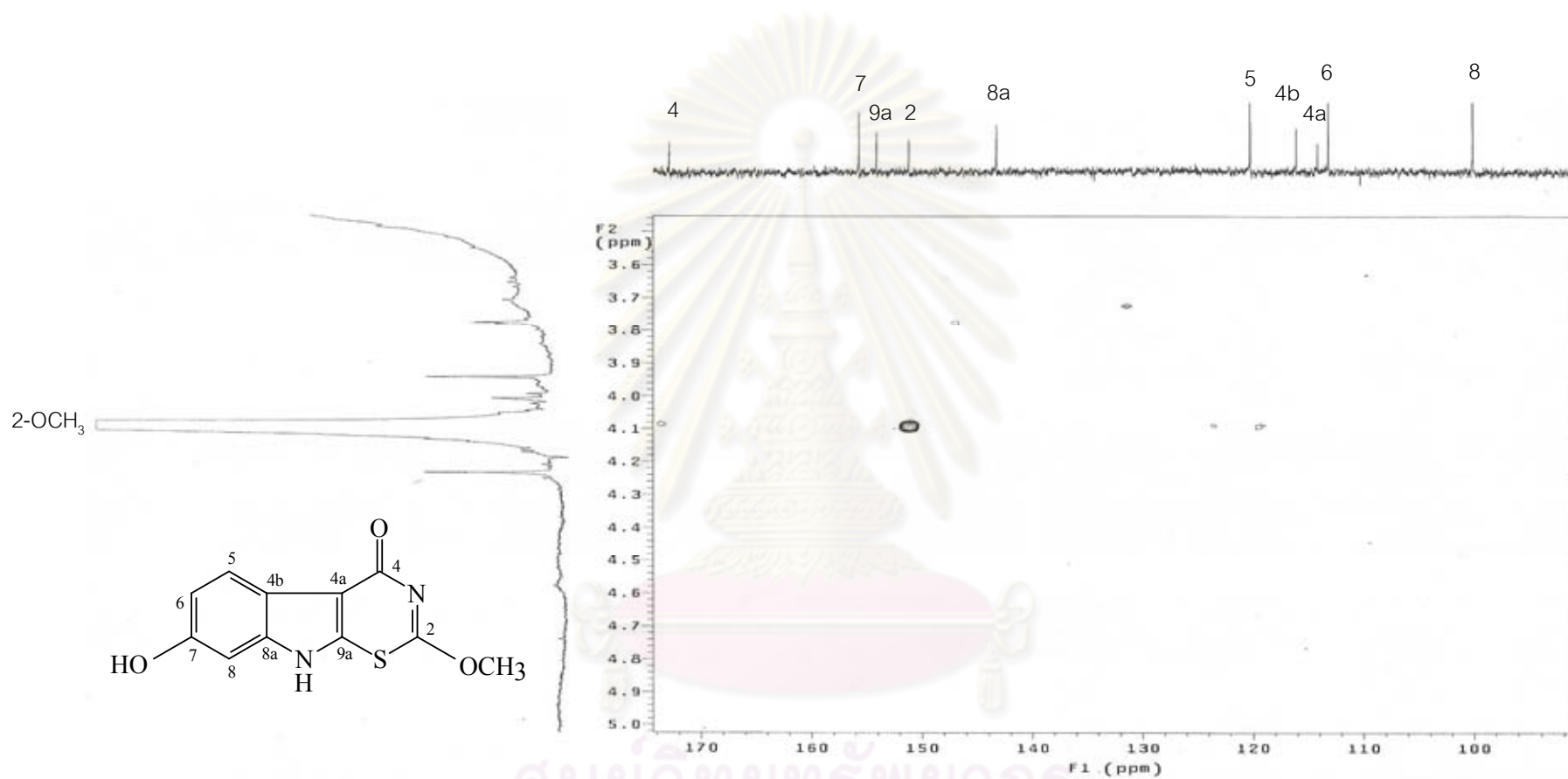
Figure 30d. HMBC Spectrum of compound MS-5 (expansion between δ_H 3.5-5.0 ppm, δ_C 90-175 ppm)
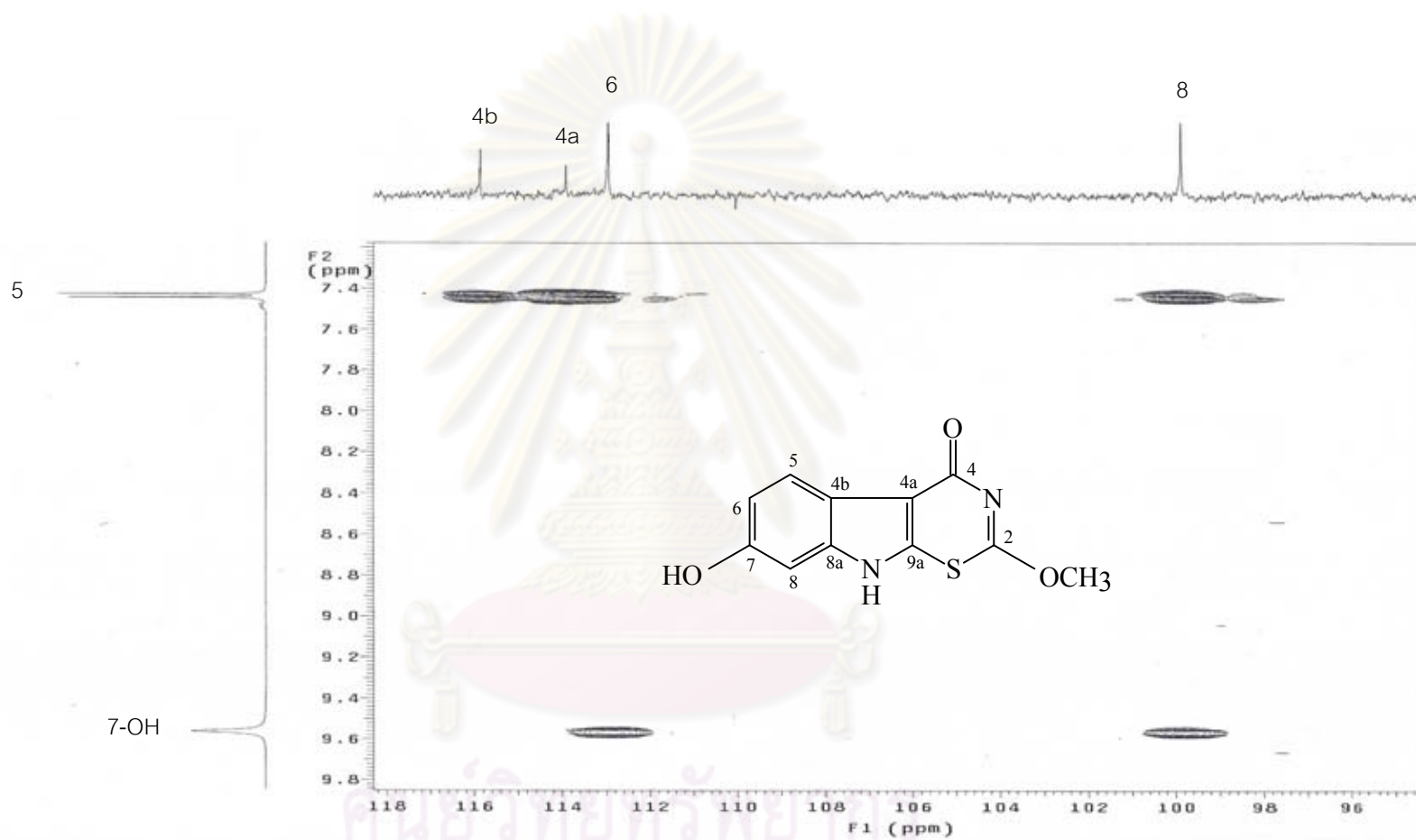
Figure 30e. HMBC Spectrum of compound MS-5 (expansion between δ_H 7.2-9.8 ppm, δ_C 94-118 ppm)

118
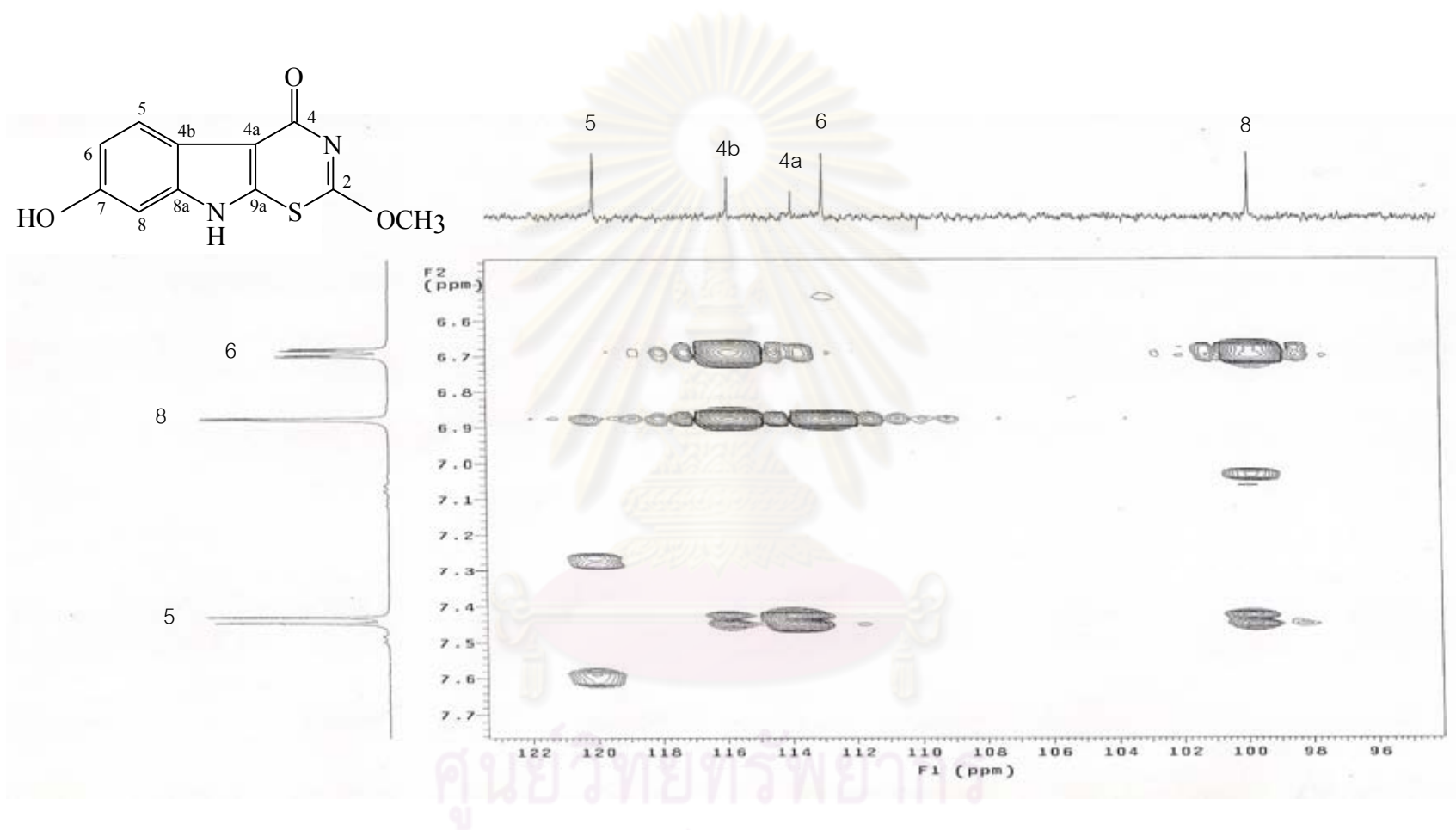
Figure 30f. HMBC Spectrum of compound MS-5 (expansion between δ_H 6.5-7.7 ppm, δ_C 94-124 ppm)
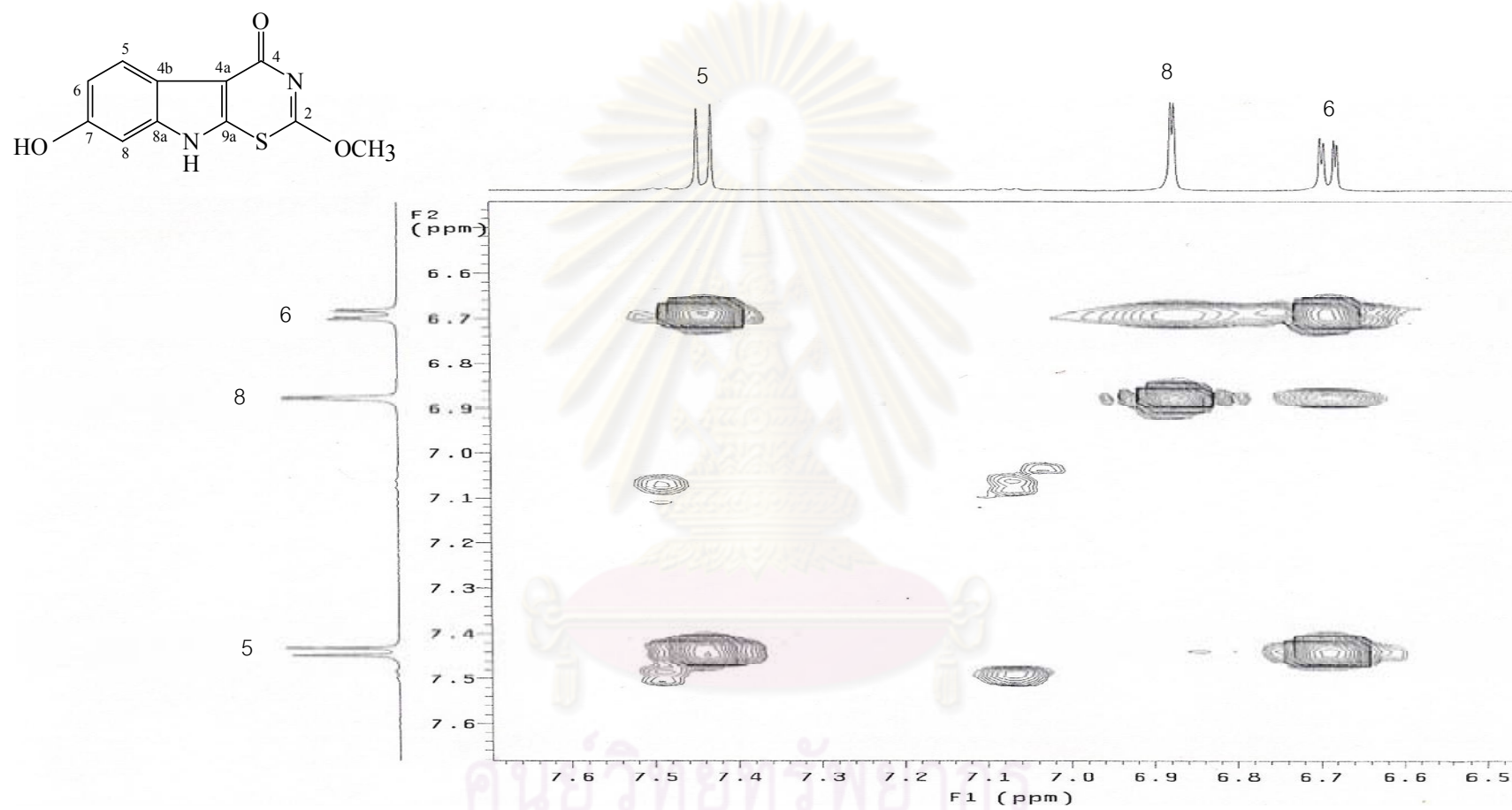
Figure 31. ^{1}H-^{1}H COSY Spectrum of compound MS-5 (expansion between δ 6.5-7.7 ppm)
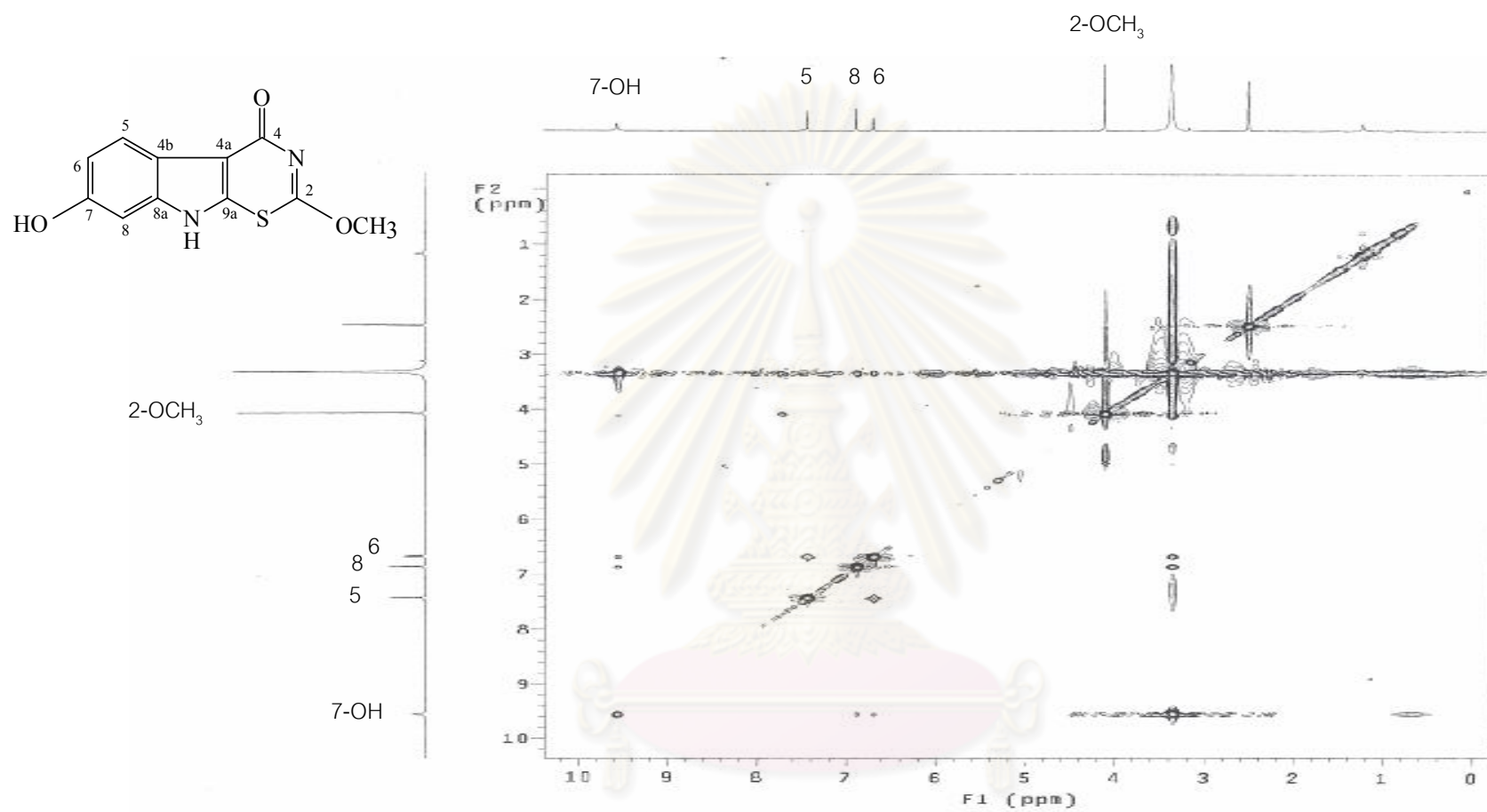
Figure 32. NOESY Spectrum of compound MS-5

VITA

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Poster presentation

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