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นางสาวพัชรา บูรณชาติ

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PHYTOCHEMICAL STUDY OF THE ROOTS OF *MORINDA ANGUSTIFOLIA* ROXB.

Miss Patchara Booranachad

A Thesis Submitted in Partial Fulfillment of the Requirements
for The Degree of Master of Science in Pharmacy in Pharmaceutical Botany

Department of Pharmaceutical Botany
Faculty of Pharmaceutical Sciences
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พัชรา บูรณชาติ: การศึกษาทางพฤกษเคมีของรากยอดิน (PHYTOCHEMICAL STUDY OF THE ROOTS OF *MORINDA ANGUSTIFOLIA* ROXB.) อาจารย์ที่ปรึกษา: รศ.ดร.เอกรินทร์ สายฟ้า, 124 หน้า. ISBN 974-17-5147-8

จากการศึกษาทางพฤกษเคมีของรากยอดิน (*Morinda angustifolia* Roxb.) สามารถแยกสารบริสุทธิ์ในกลุ่มแอนทราควิโนนได้ 3 ชนิดคือ 1-hydroxy-2-methoxy-3-formyl anthraquinone, Soranjidiol และ Morindone และ สารผสมในกลุ่มสเตียรอยด์ คือ Stigmasterol กับ β-sitosterol ซึ่งการพิสูจน์โครงสร้างทางเคมีของสารเหล่านี้ทำโดยการวิเคราะห์ข้อมูลทางสเปคโตรสโคปีประกอยด้วย EIMS UV IR และ NMR รวมทั้งการเปรียบเทียบกับข้อมูลที่มีรายงานมาก่อน

ภาควิชา เภสัชพฤกษศาสตร์ ลายมือชื่อนิสิต ……..……...……

สาขาวิชา เภสัชพฤกษศาสตร์ ลายมือชื่ออาจารย์ที่ปรึกษา……...……..

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KEY WORD: *MORINDA ANGUSTIFOLIA* ROXB./ RUBIACEAE/
ANTHRAQUINONE/ ANTHRAQUINONE IN *MORINDA* SPECIES

PATCHARA BOORANACHAD: PHYTOCHEMICAL STUDY OF THE ROOTS OF *MORINDA ANGUSTIFOLIA* ROXB. THESIS ADVISOR:ASSOCIATE PROFESSOR EKARIN SAIFAH, Ph.D. 124 PP. ISBN 974-17-5147-8

Three Anthraquinones, 1–hydroxy-2-methoxy-3-formyl anthraquinone, Soranjidiol, and Morindone and a mixture of Stigmasterol and *β*-sitosterol were isolated from the roots of *Morinda angustifolia* Roxb. The chemical structures of these compounds were elucidated through extensive analyses of their MS, UV, IR and NMR spectroscopy, as well as comparison with the previously reported data.

Department Pharmaceutical Botany Student's signature ……..…...…..

Field of study Pharmaceutical Botany Advisor's signature ……...…….

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ABBREVIATIONS

br	=	broad (for NMR spectra)
^{o}C	=	degree celsius
^{13}C NMR	=	Carbon–13 Nuclear Magnetic Resonance
$CDCl_3$	=	deuterochloroform
$CHCl_3$	=	chloroform
cm	=	centimeter
COSY	=	^{1}H–^{1}H Correlated Spectroscopy
δ	=	chemical shift
1–D	=	one dimensional
2–D	=	two dimensional
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO-d_6	=	deuterated dimethylsulfoxide
ε	=	molar absorptivity
EIMS	=	Electron Impact Mass Spectra
EtOAc	=	Ethyl acetate
eV	=	electron volt
g	=	gram
^{1}H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H–detected Heteronuclear Multiple Bond Correlation
HMQC	=	^{1}H–detected Heteronuclear Multiple Quantum Coherence
Hz	=	hertz
IR	=	Infrared
J	=	coupling constant
KBr	=	Potassium Bromide

kg	=	kilogram
λ_{max}	=	wavelength at maximum absorption
m	=	multiplet (for NMR spectra)
M^+	=	molecular ion
m/z	=	mass–to–charge ratio
MeOH	=	methanol
MHz	=	megahertz
mg	=	milligram
ml	=	milliliter
MS	=	Mass Spectrum
m.p.	=	melting point
ν_{max}	=	wavenumber at maximum absorption
nm	=	nano meter
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Correlates Spectroscopy
ppm	=	part per million
q	=	quartet (for NMR spectra)
rel. int .	=	relative intensity
s	=	singlet (for NMR spectra)
sp.	=	species
t	=	triplet (for NMR spectra)
td	=	triplet of doublet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet

CHAPTER I

INTRODUCTION

Several flowering plants in the family Rubiaceae have been used as dyestuffs as well as medicines for a long time. A lot of research works have been done in both pharmacological and phytochemical studies of Rubiaceous plants, including genus *Morinda*, which can be found from Tropical Asia to Polynesia (Perry, 1980). Backer and Bakhuizen (Backer and Bakhuizen, 1965) has described genus *Morinda* as follows:

> "Flowers in peduncled capituliform, irregularly globose or ovoid inflorescences, 4-6-merous, unisexual (but seemingly bisexual), or bisexual; bracts small; calyx-tubes entirely connate or almost so; limb very short, usually truncate, rarely with 1 or 2 leaf-like lobes (calycophylly); corolla-lobes valvate in bud, keeled inside; stamens inserted in throat or slightly lower, exsert; filaments short; anthers doesifixed below the middle; disk annular, glabrous; style dimorphic, glabrous; style-branches (partly stigmatic) 2, narrow; ovary 2-celled or incompletely 4-celled; ovule 1 per cell, attached near base of septum; fruit a 1-pyrenous, 1-seeded drupe, the fruits together forming a fleshy syncarp. Leaves opposite, penninerved, not rarely on upper side with numerous bacteriodomatia, on underside in axils of midrib and nerves with pubescent acarodomatia; stipules interpetiolar. Trees, or erect or climbing shrubs."

According to the record of Index Kewensis and its supplements there are about 150 *Morinda* species. The following eleven species are native of Thailand (Smitinand, 1980).

1. *Morinda angustifolia* Roxb. (Figures 1a-1b)

   Yo din (Trang); Salak baan (Northern); Salak paa (Central); Khoh (Karen-Mae Hong Son)

2. *M. angustifolia* Roxb. var. *scrabridula* Craib

   Charak dong, Tueng sai, Salak (Chiang Mai); Salak paa (Lampang)

3. *M. citrifolia* Linn.

   Yo baan, Yo (Central); Ma ta suea (Northern); Yae-yai (Karen-Mae Hong Son)

4. *M. coreia* Ham. [= *M. tinctoria* Roxb.]

   Salak paa, Salak luang (Northern); Yo paa (General); Khui (Phitsanulok); Khu (Karen-Kanchanaburi)

5. *M. elliptica* Ridl. [= *M. citrifolia* Linn. var. *elliptica* Hook. (Craib, 1934)]

   Yo thuean (Chumphon); Yo paa (Trang, Satun); Ka-muu-duu (Malay-Pattani); Muu duu (Malay-Narathiwat)

6. *M. pandurifolia* Ktze. [=*M. persicifolia* Williams var. *pandurifolia* Pitard (Craib, 1934)]

   Kaam Kung (Loei)

7. *M. pandurifolia* Ktze. var. *oblonga* Craib [=*M. persicifolia* Williams var. *oblonga* Pitard, *M. persicifolia* Williams (Craib, 1934)]

   Yo naa (Central, Penninsula); Yo nam (Chai Nat); Yo paa (Ang Thong); Yo paa lek (Nakhon Sawan)

8. *M. pandurifolia* Ktze. var. *tenuifolia* Craib

   Yo tia (Surat Thani)

9. *M. talmyi* Pierre [= *M. persicifolia* Williams var. *talmyi* Pitard (Craib, 1934)]

   Yo bia (Si Sa Ket); Khamin Thung (Nakhon Ratchasima)

10. *M. tomentosa* Heyne ex Roth [= *M. tinctoria* Roxb. var. *tomentosa* Hook. (Craib, 1934)]

    Yo paa, Khoh Khamin, Sakue, Sa koei, Haskoei (Northern); Talum phuk (Khon Kaen); Ta kraei (Ratchaburi); Khu yuu (Karen-Mae Hong Son, Suai); Khwoh (Karen-Kanchanaburi)

11. *M. umbellata* Linn.

    Yo yaan (Peninsular)

The plant used in this investigation was found in the northeastern Thailand. The specimen of this plant was identified to be *Morinda angustifolia* Roxb. (Figures 1a-1b), family Rubiaceae.

M. angustifolia Roxb. is a small deciduous shrub found in the eastern Himalayan and many other states of India up to an altitude of 1800 m. The roots of the plant are used for colouring agents, and condiment (Bhuyan et al., 2002). Phengklai describe *Morinda angustifolia* Roxb. as follows (Phengklai, 1988):

> "Small shrub, 0.5–2 m. high; young branches quadrangulate, sparsely scarbrous; old branches cylindrical, glabrous. Leaves simple, opposite, decussate; lanceolate oblong to oblanceolate; rather thick and scabrous both sides; cuneate base; broadly upwards then cuspidate apex; entire or undulate margin, with interpetiolar stipule. Petiole about 1.5 cm long. Flowers in a compact head, axillary. Petals 4, white, united at base into a salvev–shaped tube. Fruit aggregate, rather succulent. Disttibution : Scattered in miost deciduous and evergreen forests, in the northern part of country; altitude 800-1200 m."

A preliminary chemical investigation of this plant showed the presence of anthraquinone. The result was later confirmed by thin-layer chromatography.

Figure 1a. *Morinda angustifolia* Roxb.

Figure 1b. *Morinda angustifolia* Roxb.

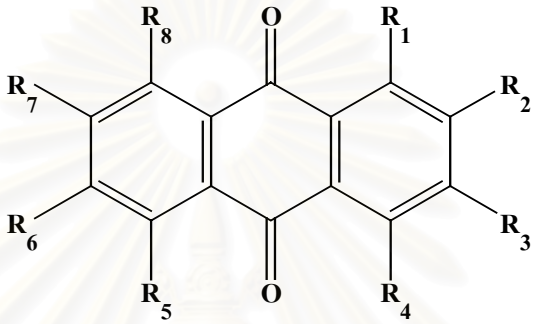
Various species of *Morinda* have been know as dye plants since the ancient time. In India, cotton, wool and silk were coloured with *Morinda* root-dyes, which are known under the name “al”, “ach” “surangi” etc. *M. citrifolia* Linn. and *M. coreia* Ham. Are considered to be the chief sources of “al” dye. Some of the other species of this genus, particulary *M. bracteata* Roxb. , *M. tomentosa* Heyne ex Roth and *M. umbellata* Linn. were also explorted for dyes. For the Javanese dyeing industry, *M. citrifolia* Linn. is cultivated. *Morinda* species yield dyes which give permanent shades of red, purple and chocolate which are produced on moranted cotton, silk or wool, the shades being fast to soap (Bhuyan *et al*., 2002).

Morinda species are well-known medicinal plants in many countries. *Morinda morindoides* is one of the most popular medicinal plants in the Democratic Republic of Congo. Its traditional use to against rhumatic pains (Cimanga *et al*., 2003). Its leaves used as antidiarrhoeic (Tona *et al*., 1998). The roots of *Morinda officinali*s used as a Chinese traditional Yang-tonic drug (Cui *et al*., 1995). In Thailand,

the root of *M. citrifolia* Linn. is used as a cathartic drug, fruits are used to stop vomiting (Pongboonrod, 1971). In Bombay, the leaves are used as a healing application to wounds and ulcers, and are administered internally as a tonic and febrifuge. In Indochina, the fruits are used as medicine for dysentery and asthma; it is also used as a deobstruent and emmenagogue. In Guinea, a decoction of the roots is taken as an emetic and a laxative. An infusion of the leaves is considered emollient, sedative and stomachic. The leaves are also used to relieve fever and headache. In Java, pulp of the seed-freed ripe fruits, are mashed with sugar and drink as a slightly laxative preparation. The rhizome and root of *Morinda parvifolia* Bartl. are known as "Hong-Zhu-Teng" or Bai-Yen-Teng" in Chinese Folkeore as herbal remedies for the treatment of human bronchitis and wooping cough (Chang *et al*., 1982). The wood and leaves of *Morinda* species are reported to have innumerable medicinal uses as purgative, antiseptic and also in asthma and dysentery types of ailments (Bhuyan, *et al*. 2002). An extract of *M. citrifolia* Linn. var. *bracteata* (Roxb.) Hook. is taken to relieve aching bones (Perry, 1980). A crude ethanol extract and hexane fraction of *Morinda citrifoli*a Linn. show antitubercular activity (Saludes *et al*., 2002). The stem-bark of *M. lucida* Linn. yields anthraquinones as well as alkaloids, which posses anti-neoplastic property in mice. *M. lucida* Linn. is locally abundant in Nigeria, where it has a reputation for antipyretic and antimalarial properties (Durodala, 1974). The leaves extract of this plant inhibited parasite *in vitro* (Tona *et al*., 1999). The leaves extract of *Morinda morindoides* showed potent dose-dependent anticomplementary activity (Cimanga *et al*., 1995), and antimalarial activity (Tona *et al*., 2001). The roots of *Morinda officinalis* possess hypoglycemic, and anti-oxidant properties (Soon and Tan, 2002).

The chemistry of rubiaceous plants are very interesting especially the chemistry of genus *Morinda*. The chemical study of genus *Morinda* showed the presence of anthraquinones. Previous phytochemical study of *Morinda angustifolia*

Roxb. was done in the leaves and heart wood and the isolation of aloe-emodin (1), morindone (2) and rhein (3) were reported (Rao *et al*., 1978), whereas the roots of *M. angustifolia* Roxb. was recently done by Bhuyan and Saikia (Bhuyan and Saikia, 2003) and the isolation of aloe-emodin (1), morindone (2), rhein (3), emodin (4) and morindonin (5) were reported.



No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1.	OH	H	CH_2CH_3	H	H	OH	H	OH
2.	OH	CH_3	H	H	OH	OH	H	H
3.	OH	H	COOH	H	H	H	H	OH
4.	OH	H	CH_3	H	H	OH	H	OH
5.	OH	H	CH_3	H	H	H	O-prime-H_6	OH

It is the purpose of this investigation to studies, the chemical constituents of the roots of *Morinda angustifolia* Roxb. The results may serve as a additional information on the phytochemical nature of plant in this family. Moreover, some isolated compounds would provided information to clarify their structures which lead to the valuable information in the field of chemotaxonomy.

CHAPTER II

HISTORICAL

1. Distribution of the anthraquinone compounds in natural sources

Anthraquinone compounds are the largest group of natural quinones. They are widely distributed in the plant kingdom and some animals such as in insects (*Coccidae* only) and in feather star (*Crinoidea*) (Shibata, 1967). They are frequently found in lichens such as *Caloplaca* which are remarkable by the yellow to red colour because of its anthraquinone pigments, parietinic acid and emodin (Bohman, 1969). It was reported that the first appearance of anthraquinones was found in bacteria (Thomson, 1971). In moulds, anthraquinones are mostly found in *Aspergillus* and *Penicillium*. More than twenty anthraquinones and related compounds have been isolated from *Penicillium islandicum spp*. (Shibata, 1967). The distribution of anthraquinones in higher plants are mostly in the Rubiaceae, they account for half the total number. They are located chiefly in heartwood, bark and roots, sometimes they are in stem, seeds and fruits (Thomson, 1971).

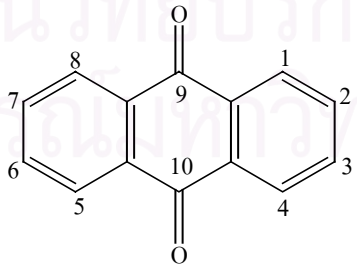
Anthraquinones can be found among the following genera of the Rubiaceae: *Morinda*, *Galiu*, *Coprosma*, *Damnacanthus*, *Hymendictyon*, *Hedyotis* (Gibbs, 1974), *Prismatomeris* (Lee, 1969) and *Coelospermun* (Thomson, 1971). There was also a report of the anthraquinones in callus cultures of *Cinchona ledgeriana* Moens Rubiaceae) (Wijnsma *et al*., 1984). The bark of *Coprosma australis* Robinson contains 17 % of its dry weight of anthraquinones. The root of *Rubia tinctorum* Linn. (madder) has been known to contain about twenty anthraquinones (Thomson, 1971). The other families also containing anthraquinone pigments are Rhamnaceae [*Rhamnus* (Gibbs, 1974), *Maesopsis* (Cumming, 1970), *Ventilago* (Cooke and Johnson, 1963)], Polygonaceae [particularly *Rumex*, *Rheum* and *Polygonum* (Gibbs, 1974)], Leguminoceae, subfamily Caesalpiniaceae [*Cassia* (Takido, 1958)], subfamily Papillionaceae [*Abrus* (Gibbs, 1974)], Bignoniaceae [*Tabebuia avellanedae* Lor. Ex

Griseb. Verbenaceae [*Tectona grandis* Linn. (Ahluwalia and Seshadri, 1957)] and Scrophulariaceae [*Digitalis* species (Gibbs, 1974)]. Anthraquinones are also found among monocotyledons especially in the Liliaceae and Xyridaceae. *Aloe* (Rheede, 1963), *Asphodeline*, *Asphodelus*, *Bulbine*, *Enemerus* (Rheede, 1964) and *Polygonatum* (Gibbs, 1974) represented the genera of the Liliaceae which contained anthraquinones, as well as *Xyris indica* Linn. (Ruangrungsri and Tantivatana, 1980) and *X. semifuscata* Baker (Fournier *et al*., 1975) of the Xyridaceae. Some anthraquinones are found in Anacardiaceae, Apocynaceae, Asclepiadaceae, Caryophyllaceae, Compositae, Ericaceae, Euphorbiaceae, Lythraceae, Rhizophoraceae, Saxifragaceae (Gibbs. 1974) and Solanaceae (Knapp, 1972).

2. Chemical Nature of Anthraquinone Compounds

Anthraquinone itself has been obtained from several natural sources (Thomson, 1971). Most of the anthraquinones are red yellow or orange yellow colouring matter, varying from yellow to brown. The anthraquionone crystals are high–melting point compounds, they are soluble in oganic solvents. Anthraquinones composes of three benzene rings having the quinoid doublebond. Most of the naturally occurring anthraquinones are hydroxylated at the C–1 position.

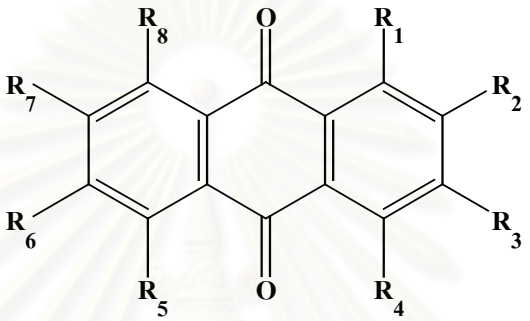
The fundamental structure (6) and the numbering system of anthraquinones is shown below.



(6)

The anthraquinones can be classified into two groups according to their biosynthetic pathway. The first group consists of anthraquinones with substitution in ring A and C. They are found in fungi and higher plants. In fungi, emodin (4), endocrocin (7) and islandicin (8) were found. In higher plants anthraquinones are

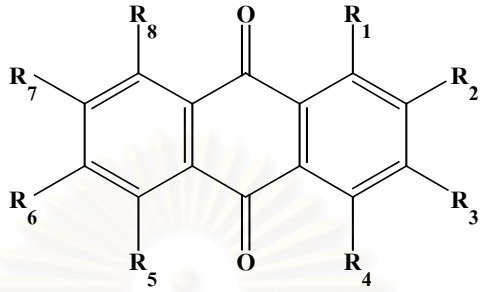
distributed in Leguminoceae (subfamily Caesalpiniaceae and Papillionaceae), Polygonaceae and Rhamnaceae e.g. emodin (4), aloe–emodin (1) and chrysophanol (9). The other group consists of anthraquinone with substitution only in the ring C. They are found mainly in Bignoniaceae, Rubiaceae, Scrophulariaceae and Verbenaceae e.g. alizarin (10), lucidin (11) and rubiadin (12) (Robinson, 1967) as shown below.



No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
7.	OH	COOH	CH_3	H	H	OH	H	OH
8.	OH	H	CH_3	OH	H	H	H	OH
9.	OH	H	CH_3	H	H	H	H	OH
10.	OH	OH	H	H	H	H	H	H
11.	OH	CH_2OH	OH	H	H	H	H	H
12.	OH	CH_3	OH	H	H	H	H	H

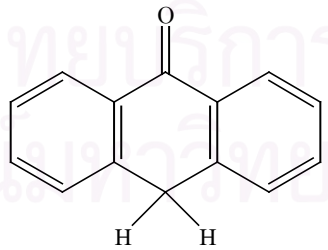
The anthraquinones actually existed in plants are apparently in several forms. They are often found as anthraquinone glycosides rather than hydroxylated anthraquinones or aglycones. Reports of the appearance of free anthraquinones must be regarded cautiously. Many anthraquinones occur as glycosides with the sugar residue linked through one of the phenolic hydroxyl groups. Several different sugars are found in such glycosides. For example, alizarin (10) occurs as a 3–glucoside (13) in *Rubia tinctorum* Linn. (madder) and as a 3–primeveroside (14) in *Galium* species; and

morindone (2) occurs as a 6–rutinoside (15) in *Coprosma australis* Robinson and as a 6–primeveroside (16) in *Morinda persicaefolia* Ham. (Robonson, 1967).

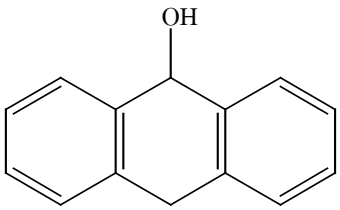


No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
13.	OH	OH	glu	H	H	H	H	H
14.	OH	OH	Prime-H_6	H	H	H	H	H
15.	OH	CH_3	H	H	OH	O-rutinose	H	H
16	OH	CH_3	H	H	OH	O-prime-H_6	H	H

The aglycones of several anthraquinone glycosides may exist naturally in reduced forms, one of which is anthrone (17). The sugar in these reduced glycosides may be linked as usual through phenolic oxygens in the outside ring or they may be attached at C–9 of the enol form of anthrone (17), anthranol (18) (Robinson, 1967).



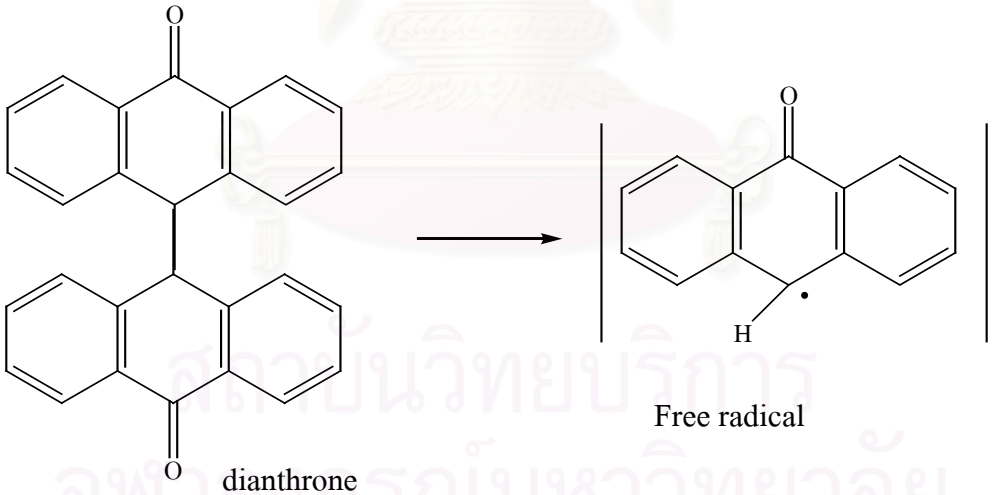
(17)



(18)

Enzymatic or chemical hydrolysis followed by oxidation of anthranol glycoside would give anthrone and anthraquinone. If the sugar is linked at some other positions except C–9 and C–10, anthranol glycosides may be directly oxidised to anthraquinone glycosides. Thus, the anthraquinone glycosides are divided in to two types. One is O–glycoside, the sugar links at the phenolic oxygen. The other is C–glycoside with sugar attached through carbon–carbon bond.

A group of glycosides is known as the sennosides. They are the glycosides of sennidin dianthrone. Sennosides A and B are, the 8, 8'–diglucosides of rhein–rhein dianthrone, and sennosides C and D are the corresponding diglucosides of aloe–emodin rhein dianthrone. The dianthrone carbon–carbon bond is liable to oxidative cleavage far more easily than the normal carbon–carbon bond. This is due to the stability of the resulting anthrone radical. Oxidation is carried out by using a free radical oxidising agent such as ferric chloride as shown below.

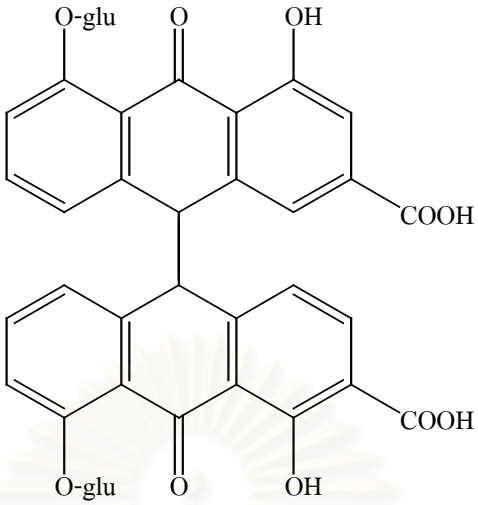


dianthrone

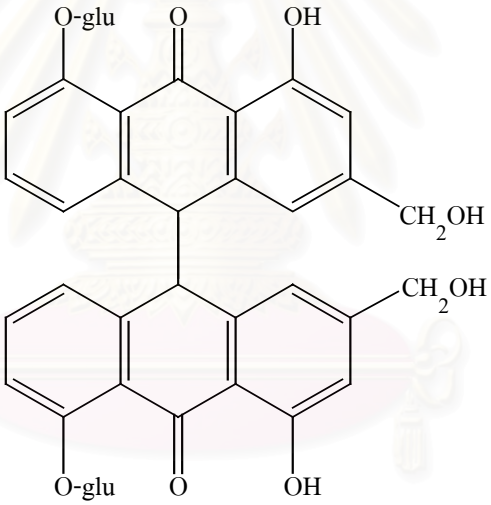
Free radical

Free radical oxidation of dianthrone

The difference between the aglycone of sennosides A and B (19) is one of optical activity and the same relationship can be applied for the aglycones of sennosides C and D (20).



(19)



(20)

Anthraquinones can be detected by the Borntrager test in which an organic solution containing the test material is shaken with an aqueous base. In bases, the anthraquinone can from phenolate type ions which are coloured. The visible result of the test in which the basic layer goes a cherry red and the intensity of the colour can be used as a measure of the amount of anthraquinone in the original material. Only free anthraquinone give a positive Borntrager test and this fact can be used to distinguish between the O- and C–glycosides, the O–glycosides being hydrolyzed to free anthraquinones by reflux with diluted hydrochloric acid while the C–glycosides release

the free anthraquinones only after oxidative cleavage. The colours given with alcoholic magnesium acetate solution are characteristic of different hydroxylation patterns (Robinson, 1967). Compound containing, two hydroxyl groups in the ortho position e.g. alizarin, exhibit a violet colour, those with two in the meta position e.g. emodin, give an orange–red or pink colour, and those with two in para position e.g. quinizarin, produce a purple. These colour reactions are specific and stable .

The most important group of laxative drugs used todays is the group of the plants products derived from materials that contain anthracene derivatives. The active conatituents are anthraquinone compounds containing phenolic groups, either free, as methyl ethers, or as glycosides. The free anthraquinones or glycosides are ineffective, and the pharmacologically important compounds are free anthranols. Anthraquinones are active as cathartics only because they are reduced to anthranols by intestinal bacteria (Robinson, 1967).

3. Biogenesis of Anthraquinone Compounds

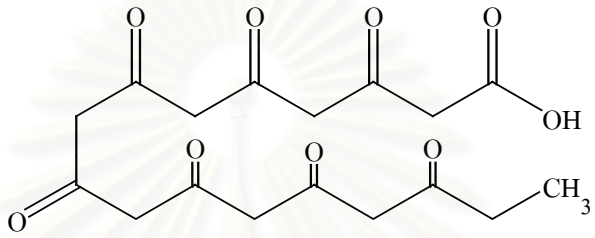
Anthraquinones are derived from a few key intermediate. principally acetate, shikimate and mevalonate by a sereis of reactions which lead to the formation of benzenoid compounds, they arise by at least two biosynthetic routes. Fungal anthraquinones such as emodin (4) and chrysophanol (9) bear structures in accord with the acetate hypothesis, while the anthraquinones found in higher plants, for example alizarin (10), have biosynthetic route via shikimate–mevalonate pathway.

The two possible biogenetic pathways of the anthraquinone compounds are as follows:

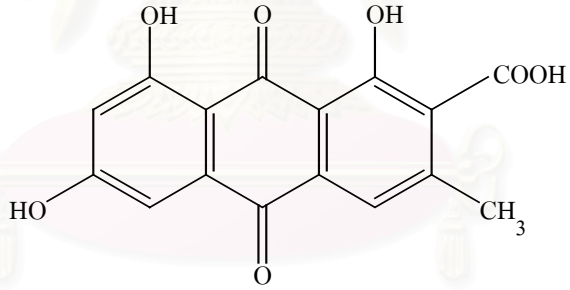
3.1 Acetate–Malonate Pathway (Thomson, 1971)

The majority of the anthraquinones which are assumed to be elaboratorated by the acetate–malonate pathway is confined to emodin pattern. They are arised by

suitable folding and condensation of a polyketide chain derived from eight acetate units, was shown below. Numerous variations of this basic structure exist, resulting from O–methylation, side–chain oxidation, chlorination, dimerization and the introduction or omission of nuclear hydroxyl groups, while in endocrocin (7) the terminal carboxyl group is retained.



Poly – B – ketomethylene compound



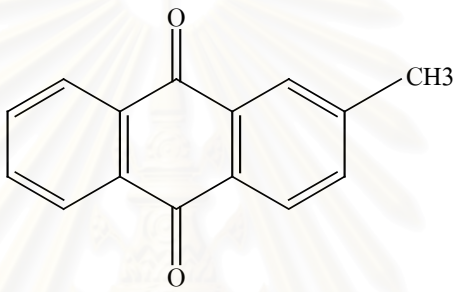
Endocrocin

Emodin can be found in higher plants, it lacks only the carboxyl residue of endocrocin, a ccomponent of the lichen *Nephromopsis endocrocea* Asahina (Asahina and Fujikawa, 1935), but bears the same pattern of oxidation.

Bacterial and fungal anthraquinones have their formation *via* the acetate–malonate pathway. As such typical fungal anthraquinones as emodin (4) and chrysophanol (9) are also found in higher plants. Leistner and Zenk (Leistner and Zenk, 1969) have proved that chrysophanol is produced in *Rumex alpinus* Linn. and *Rhamnus frangula* Linn. *via* the acetate–malonate route.

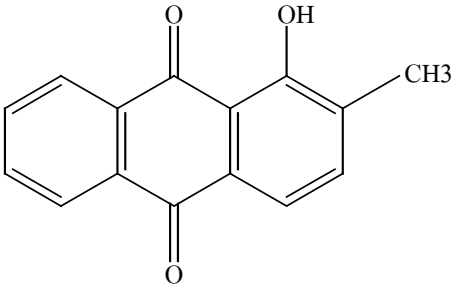
3.2 Shikimate–Mevalonate Pathway (Thomson, 1971)

Anthraquinones which are found in some higher plants especially in the order Tubiflorales have shikimate–mevalonate biosynthetic route. They are substituted in only one benzenoid ring (ring C) and may be totally devoid of a carbon side chain or hydroxl groups in ring A e.g. alizarin (10) and tectoquinone (21). The majority of these anthraquinones occur in the Rubiaceae subfamily Rubioideae and, to a lesser extent, in the Bignoniaceae and Verbeneceae.

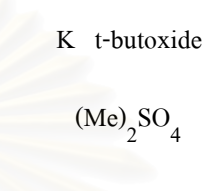


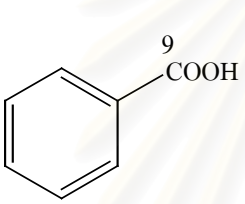
(21)

Labelled precursors, carboxyl–^{14}C–D–shikimic acid, in *Rubia tinctorum* Linn. roots, led to labelled alizarin. The distribution of radioactivity in the alizarin molecule was determined by degradation of the alizarin dimethylether which yielded benzoic acid (ring A plus C–atom 9) and veratric acid (ring C plus C–atom 10). The result of this degradation showed that the carboxyl group of shikimic acid is exclusively incorporated into C–atom 9 of alizarin.

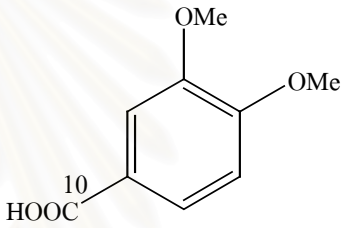


Alizarin





Benzoic acid



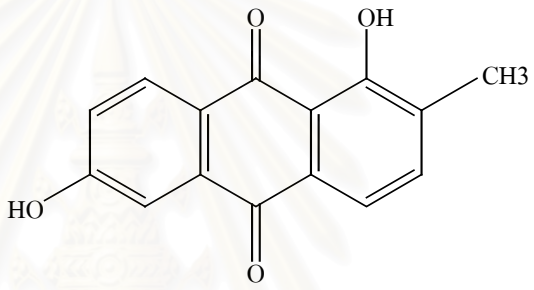
Veratric acid

After ^{14}C–2–glutamaic acid feeding, it seemed that C–2 of glutamic acid give rise specifically to C–10 of naphthalene or alizarin anthraquinone. This naphthalene could be 1, 4–dihydroxy–2–naphthoic acid which is linked to r, r–dimethylallyl pyrophosphate derived in turn from mevalonic acid, in the meta position to C–9 of alizarin. The latter observation emerges from the fact that activity from C–5 mevalonic acid is specially incorporated into C–4 of alizarin so suggesting that C–1 to C–4 are derived from mevalonic acid by way of r, r–dimethylallyl pyrophosphate. Decarboxylation and ring C closure would lead to anthraquinone such as alizarin (Leistner, 1973).

Leistner (Leistner, 1973) has shown the biosynthesis of alizarin in *Rubia tinctorum* Linn. by using tracer technique. Specific incorporation of labels from carboxyl–^{14}C–DL–mevalonic acid, 2–^{14}C–D–GLUTAMIC ACID AND 5–^{14}C–DL–mevalonic acid suggests that these compounds provide the skeleton of alizarin.

Experimental data indicate that β–keto–glutaric acid or its derivative combines with shikimic acid, chorismic acid, or phrephenic acid to give O–succinylbenzoic acid which is then transformed to a nonsymetrical 1, 4–naphthaquinone intermediate, and r, r–dimethylallyl pyrophosphate is then attached. Ring closure and futher modification lead to alizarin.

Morindone (2) and soranjidiol (22) anthraquinones of *Morinda citrifolia* Linn. are hydroxylated in both ring A and Ring C.



(22)

Experiments Carried out by Leistnet (Leistner, 1973) showed that anthraquinones in morindone are derived from shikimic acid via O–succinylbernzoic acid as the same biosynthetic pathway as alizarin. The hydroxyl groups attached to ring A are introduced at the latter stage of biosynthesis and are not derived from hydroxyl groups of shikimic acid.

The occurrence of anthraquinones in the plants genus *Morinda* were listed in the table 1.

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda*

Plant and Compound	Plant Part	Reference
Morinda angustifolia Roxb.		
Aloe-emodin (1)	Leaves	Roa *et al*., 1978
	Roots	Bhuyan and Saikia., 2003
Emodin (4)	Roots	Bhuyan and Saikia., 2003
Morindone (2)	Leaves	Roa *et al*., 1978
	Roots	Bhuyan *et al.*, 2002
	Roots	Bhuyan and Saikia., 2003
Moridonin (5)	Roots	Bhuyan and Saikia., 2003
Rhein (3)	Heart wood	Roa *et al*., 1978
	Roots	Bhuyan and Saikia., 2003
*M. citrifolia*Linn.		
1,3,5,7-tetracetoxy-2,6-dimethyl-anthraquinone (23)	Cell suspension	Inouet *et al*., 1981
1-acetoxy-2-methylanthraquinone (24)	Cell suspension	Inouet *et al*., 1981
1-acetoxyanthraquinone (25)	Cell suspension	Inouet *et al*., 1981
2-acetoxyanthraquinone (26)	Cell suspension	Inouet *et al*., 1981
2-acetoxymethyl-1,3,6-triacetoxyanthraquinone (27)	Cell suspension	Inouet *et al*., 1981
2-methyl–3,5,6-trihydroxy anthraquinone (28)	Cell suspension	Inoue *et al*., 1981

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
M. citrifolia Linn.		
2-methyl-3,5,6-trihydroxyanthraquinone-6-*β*-primeveroside (29)	Cell suspension	Inouet *et al*., 1981
Tactoquinone (21)	Cell suspension	Inouet *et al*., 1981
3-acetoxy-2-methylanthraquinone (30)	Cell suspension	Inouet *et al*., 1981
3-hydroxymorindone (31)	Cell suspension	Inoue *et al*., 1981
3-hydroxymorindone-6-*β*-primeveroside (32)	Cell suspension	Inouet *et al*., 1981
5,6-dihydroxylucidin (33)	Cell suspension	Inoue *et al*., 1981
5,6-dihydroxylucidin-3-*β*-primeveroside(34)	Cell suspension	Inouet *et al*., 1981
Alizarin (10)	Cell suspension	Inouet *et al*., 1981
	Cell suspension	Leistner, 1973
	Cell suspension	Leistner, 1975
	Heartwood	Balakrishma *et al*., 1961
Damnacanthal (35)	Heartwood	Balakrishma *et al*., 1961
	Root	Hiramatsu *et al*., 1993
	Root	Hiwasa *et al*., 1999

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
M. citrifolia Linn.		
Diacetylalizarin (36)	Cell suspension	Inouet *et al*., 1981
Lucidin (11)	Cell suspension	Inouet *et al*., 1981
Lucidin-3-*β*-primeveroside (37)	Cell suspension	Inouet *et al*., 1981
Lucidin–ω–ethyl ether (38)	Cell suspension	Leistner, 1975
Morindone (2)	Cell suspension	Inouet *et al*., 1981
	Cell suspension	Leistner, 1975
	Heartwood	Balakrishma *et al*., 1961
	Root bark	Balakrishma *et al*., 1960
	Cell suspension	Leistner, 1973
Morindone-6-*β*-primeveroside (39)	Cell suspension	Inouet *et al*., 1981
Morindonin (5)	Root bark	Balakrishma et al., 1960
Nordamnacanthal (40)	Cell suspension	Inouet *et al*., 1981
	Heartwood	Balakrishma et al., 1961
	Cell suspension	Leistner, 1975

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
M. citrifolia Linn.		
Rubiadin (12)	Cell suspension	Inouet *et al*., 1981
	Cell suspension	Leistner, 1975
Rubiadin-1- methyl ether (41)	Heartwood	Balakrishma et al., 1961
Morinda elliptica.		
1-hydroxy-2-methylanthraquinone (42)	Root	Ismail *et al*., 1997
2-formyl-1-hydroxy anthraquinone (43)	Root	Ismail *et al*., 1997
	Root	Ali *et al*., 2000
alizarin-1-methyl ether (44)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Damnacanthal (35)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Lucidin–ω–methyl ether (45)	Root	Ismail *et al*., 1997
Morindone	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Morindone-5-methyl ether (46)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Nordamnacanthal (40)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Rubiadin (12)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
Morinda elliptica.		
Rubiadin-1-methyl ether (41)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Soranjidiol (22)	Root	Ali *et al*., 2000
	Root	Ismail *et al*., 1997
Morinda longifloraa.		
Rubiadin-1-methyl ether (33)	Root	Paris and Abiusso, 1958
Morinda lucida Benth.		
1,2-dihydroxy-3-methyl anthraquinones (47)	Root	Sittie *et al*., 1999
1,3-dihydroxy-2-ethyl ether anthraquinones (48)	Root	Sittie *et al*., 1999
Digitolutein (42)	Stem	Adesogan, 1973
1-methoxy-2-hydroxy-3-methyl anthraquinones (49)	Root	Sittie *et al*., 1999
Digiferruginol (50)	Stem	Adesogan, 1973
Rubiadin-1-methyl ether (41)	Root	Sittie *et al*., 1999
1-methoxy-2-methyl-4-hydroxy anthraquinones (51)	Root	Sittie *et al*., 1999
2-aldehyde-3-hydroxy anthraquinone (52)	Root	Sittie *et al*., 1999
2-hydroxymethyl-3-hydroxy anthraquinone (53)	Root	Sittie *et al*., 1999
1-methoxy-2-methyl-4-hydroxy anthraquinones (51)	Root	Sittie *et al*., 1999
2-aldehyde anthraquinone (54)	Stem	Adesogan, 1973

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
Morinda lucida Benth .		
Tectoquinone (35)	Stem	Adesogan, 1973
3-hydroxyanthraquinone–2–carboxaldehyde (58)	Stem	Demagos *et al*., 1981
Alizarin-1-methyl ether (44)	Stem	Adesogan, 1973
Damnacanthal (35)	Root	Sittie *et al*., 1999
	Stem	Adesogan, 1973
	Stem bark, Root	Koumaglo *et al*., 1992
Digitolutein (49)	Stem bark, Root	Koumaglo *et al*., 1992
Nordamnacanthal (40)	Stem	Adesogan, 1973
Pseudopurpurin (55)	Stem	Adesogan, 1973
Rubiadin (12)	Stem	Adesogan, 1973
Rubiadin-1-methyl ether (41)	Stem	Adesogan, 1973
	Stem bark, Root	Koumaglo *et al*., 1992
Soranjidiol (22)	Root	Sittie *et al*., 1999
2-hydroxyanthraquinone-3-aldehyde (56)	Heart wood	Demagos *et al*., 1981
Soranjidiol (22)	Stem	Adesogan, 1973
Morinda officinalis		
1,6-dihydroxy-2,4-dimethoxy anthraquinone (57)	Roots	Yoshikawa *et al*., 1995

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
Morinda officinalis		
1,6-dihydroxy-2-methoxy anthraquinone (58)	Roots	Yoshikawa *et al*., 1995
1-hydroxy anthraquinone (59)	Roots	Yoshikawa *et al*., 1995
1-hydroxy-2,3-dimethyl anthraquinone (60)	Roots	Yoshikawa *et al*., 1995
Alizarin-2-methyl ether (61)	Roots	Yoshikawa *et al*., 1995
1-hydroxy-2-methyl anthraquinone (42)	Roots	Yoshikawa *et al*., 1995
1-hydroxy-3-hydroxymethyl anthraquinone (62)	Roots	Yoshikawa *et al*., 1995
Tectoquinone (21)	Cortex	Li *et al*., 1991
Alizarin-1-methyl ether (44)	Roots	Yoshikawa *et al*., 1995
Lucidin-w-methyl ether (45)	Roots	Yoshikawa *et al*., 1995
Physcion (67)	Roots	Yoshikawa *et al*., 1995
Rubiadin (12)	Roots	Yang *et al*., 1992
Rubiadin-1-methyl ether (41)	Cortex	Li *et al*., 1991
	Roots	Yoshikawa *et al*., 1995
Tectoquinone (21)	Roots	Yoshikawa *et al*., 1995
Morinda parvifolia Bartl .		
1-hydroxy-6-hydroxymethyl anthraquinone (63)	Rhizome and Root	Chang *et al*., 1984
1-hydroxy-7-hydroxymethyl anthraquinone (64)	Rhizome and Root	Chang *et al*., 1984
2-hydroxymethyl anthraquinone (65)	Rhizome and Root	Chang *et al*., 1984

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

Plant and Compound	Plant Part	Reference
Morinda parvifolia Bartl .		
Alizarin-1-methyl ether (44)	Rhizome and Root	Chang *et al*., 1984
	Root	Chang et al., 1982
Digiferruginol (51)	Rhizome and Root	Chang *et al*., 1984
Lucidin-ω-ethyl ether (38)	Rhizome and Root	Chang *et al*., 1984
Morindaparvin-A (66)	Rhizome and Root	Chang *et al*., 1984
	Root	Chang et al., 1982
Morindaparvin-B (67)	Root	Chang et al., 1982
*Morinda persicaefolia*Buch.-Ham.		
Morindone-6-primeveroside (16)	Root bark	Rao *et al*., 1977
Morindoin (5)	Stem	Paris and Nguyen, 1954
Morinda tinctoria Roxb .		
Damnacanthal (35)	Heartwood	Eswaran *et al*., 1979
	Root	Mishra and Gupta, 1982
Morindone (2)	Heartwood	Eswaran *et al*., 1979
	Root bark	Rao *et al*., 1977
	Root bark	Murti et al., 1959
Morindone-6-O-primeveroside (16)	Root bark	Rao *et al*., 1977
Morindonin (5)	Root bark	Murti et al., 1959

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

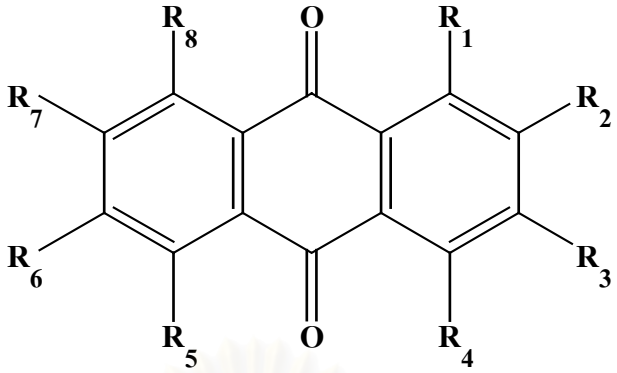
Plant and Compound	Plant Part	Reference
Morinda tinctoria Roxb .		
Nordamnacanthal (40)	Heartwood	Eswaran *et al*., 1979
	Root	Mishra and Gupta, 1982
Morinda tomentosa Heyne ex Roth.		
Alizarin-1-methyl ether (44)	Root bark	Rao and Rao, 1983
Morindone (2)	Stem bark	Roa and Reddy, 1977
Morindone–6–O-primeveroside (16)	Stem bark	Roa and Reddy, 1977
Rubiadin (2)	Root bark	Rao and Rao, 1983
Soranjidiol (22)	Root bark	Rao and Rao, 1983
Morinda umbellata Linn.		
1-Hydroxy–2–methyl anthraquinone (42)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
2–Hydroxyanthraquinone (68)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
2–Methoxyanthraquinone (69)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
2–Methoxyanthraquinone (69)	Root	Burnett and Thomson, 1968

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

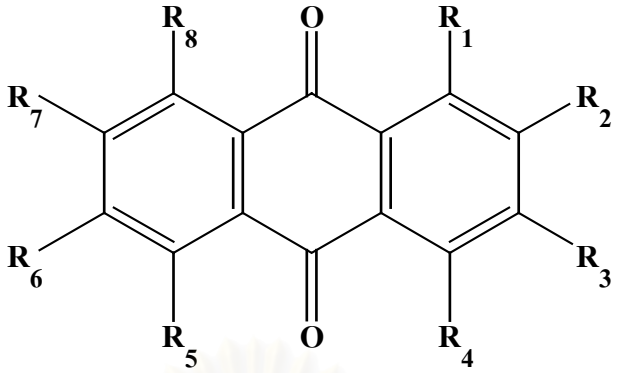
Plant and Compound	Plant Part	Reference
Morinda umbellata Linn.		
Alizarin (10)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
Alizarin–1–methyl ether (44)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
Alizarin–2–methyl ether (61)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
Lucidin (11)	Root	Burnett and Thomson, 1968
Munjistin (70)	Root	Burnett and Thomson, 1968
Rubiadin (12)	Root	Burnett and Thomson, 1968
	Stem	Burnett and Thomson, 1968
Rubiadin–1–methyl ether (41)	Stem	Burnett and Thomson, 1968
Tectoquinone (21)	Stem	Hui and Yee, 1967
Xanthopurpurin (78)	Root	Burnett and Thomson, 1968

Table 1 : The occurrence of anthraquinones in the plants genus *Morinda* (continued)

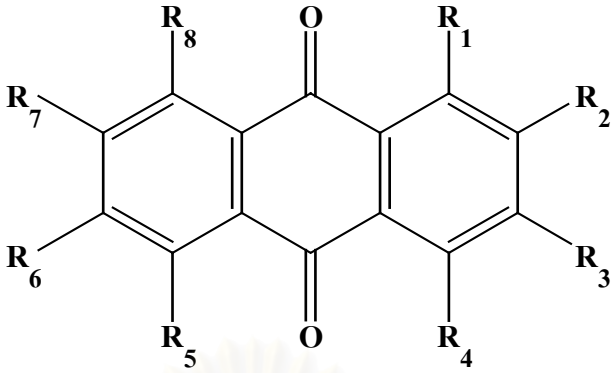
Plant and Compound	Plant Part	Reference
Morinda umbellata Linn.		
Xanthopurpurin (78)	Stem	Burnett and Thomson, 1968



No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
22.	OH	CH_3	H	H	H	OH	H	H
23.	OAc	H	CH_2CH_3	H	H	OH	H	OH
24	OAc	CH_3	H	H	H	H	H	H
25	OAc	H	H	H	H	H	H	H
26	H	OAc	H	H	H	H	H	H
27	OAc	CH_2OAc	OAc	H	H	OAc	H	H
28	H	CH_3	OH	H	OH	OH	H	H
29	H	CH_3	OH	H	OH	O-prime-H_6	H	H
30	H	CH_3	OAc	H	H	H	H	H
31	OH	CH_3	OH	H	OH	OH	H	H
32	OH	CH_3	OH	H	OH	O-prime-H_6	H	H
33.	OH	CH_2OH	OH	H	OH	OH	H	H
34.	OH	CH_2OH	O-prime-H_6	H	OH	OH	H	H
35.	OCH_3	COH	OH	H	H	H	H	H
36	OAc	OAc	H	H	H	H	H	H
37	OH	CH_2OH	O-prime-H_6	H	H	H	H	H
38	OH	CH_2-O-CH_2CH_3	OH	H	H	H	H	H
39	OH	CH_3	H	H	OH	O-prime-H_6	H	H
40.	OH	CHO	OH	H	H	H	H	H



No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
41.	OCH_3	CH_3	OH	H	H	H	H	H
42	OH	CH_3	H	H	H	H	H	H
43	OH	CHO	H	H	H	H	H	H
44	OCH_3	OH	H	H	H	H	H	H
45	OH	CH_2OCH_3	OH	H	H	H	H	H
46	OH	CH_3	H	H	OCH_3	OH	H	H
47	OH	OH	CH_3	H	H	H	H	H
48	OH	OCH_2CH_3	OH	H	H	H	H	H
49	OCH_3	OH	CH_3	H	H	H	H	H
50	OCH3	CH_3	H	H	H	H	H	H
51	OCH3	CH_3	H	OH	H	H	H	H
52	H	CHO	OH	H	H	H	H	H
53	H	CH_2OH	OH	H	H	H	H	H
54	H	CHO	H	H	H	H	H	H
55	OH	CO_2H	OH	OH	H	H	H	H
56	H	OH	CHO	H	H	H	H	H
57	OH	OCH_3	H	OCH_3	H	OH	H	H
58	OH	OCH_3	H	H	H	OH	H	H
59	OH	H	H	H	H	H	H	H
60	OH	CH_3	CH_3	H	H	H	H	H



No	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
61	OH	OCH_3	H	H	H	H	H	H
62	OH	H	CH_2OH	H	H	H	H	H
63	OH	H	H	H	H	CH_2OH	H	H
65	H	CH_2OH	H	H	H	H	H	H
66	$-OCH_2O-$	H	H	H	H	H	H	H
67	OH	CH_2OH	H	H	OH	H	H	H
68	H	OH	H	H	H	H	H	H
69	H	OCH_3	H	H	H	H	H	H
70	OH	CO_2H	OH	H	H	H	H	H
71	OH	H	OH	H	H	H	H	H

CHAPTER III

EXPERIMENTAL

1. Source of plant material

The roots of *Morinda angustifolia* Roxb. used in this study was obtained from Doi Phu Kha National Park, Bo Klua district, Nan Province, Thailand. It was identified to be *Morinda angustifolia* Roxb., family Rubiaceae by compare with voucher specimens at the Botany Section, Technical Division, Department of Royal Forest, Ministry of Agriculture and co–operative, Thailand.

2. General techniques

2.1 Chromatography

2.1.1 Analytical Thin–layer chromatography (TLC)

Technique	:	One dimension ascending
Adsorbent	:	Silica gel 60 F254, (E. Merck)
Layer thickness	:	0.2 mm
Distance	:	7 cm
Temperature	:	room temperature (30-35°C)
Detection	:	1) UV light at the wavelengths of 254 and 365 nm. 2) Spraying with vanillin-sulfuric acid reagent (10% ethanolic sulphuric acid) ant heating at 110°C for 5-10 minutes. 3) Exposing to ammonia vapour.
Solvent	:	Various solvent systems depending on materials.

2.1.2 Column chromatography

Column	:	Flat bottom glass column (various diameter)
Adsorbent	:	Silica gel 60 (No. 9385, E. Merck) particle size 0.040-0.063 mm (230-400 mesh ASTM)

Packing method : Dry and wet packing

Sample loading : 1) Dry packing : The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then loaded on the top of column.

2) Wet packing : The sample was dissolved in a small amount of the eluent, then loaded on the top of column.

Solvent system : Various solvent systems depending on materials.

Detection : 1) UV light at the wavelengths of 254 and 365 nm.

2) Spraying with vanillin-sulfuric acid reagent (10% ethanolic sulphuric acid) ant heating at 110°C for 5-10 minutes.

3) Exposing to ammonia vapour.

2.1.3 Gel filtration chromatography

Gel filter : Sephadex LH-20

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 volume of the eluent.

Solvent : Various solvent systems depending on materials.

2.2 Physical properties

2.2.1 Melting point

Melting points were determined on a Fisher-Johns melting point Apparatus serial 40500105 (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) absorption spectra

Ultraviolet absorption spectra were determined on the Milton Roy Spectronic 3000 Array Spectrometer (Pharmaceutical Research Equipment Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University)

2.3.2 Infrared (IR) absorption spectra

Infrared absorption spectra were recorded as KBr disc on a Perkin Elmer Model 283 spectrophotometer. The absorption bands were reported in wave number (cm^{-1}) (Pharmaceutical Research Equipment Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University), Perkin Elmer Infrared Spectrometer Model 1760 X (Scientific and Technological Research Equipment Center, Chulalongkorn University).

2.3.3 Mass spectra (MS)

Electron impact mass spectra (EIMS) were recorded on a Mass spectrometer model Polaris Q MS 21079, Direct Probe Controller DPC 10493. (Faculty of Sciences, Mahidol University).

2.3.4 Proton and Carbon-13 Nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectra

^{1}H NMR and ^{13}C NMR spectra were obtained on JEOL. Nuclear magnetic resonance spectrometer model XL–500. (Scientific and Technological Research Equipment Center, Chulalongkorn University)

2.4. Solvent

The solvents were used as analytical grades and redistilled commercial grades.

3. Extraction procedure

The dried roots of *Morinda angustifolia* Roxb. (950 g.) were chopped and ground to powder. They were macerated eight times with methanol (2 L, 3 days each). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 50°C to yield the methanol extract as a syrupy mass (90 g).

4. Isolation procedure

The Crude Methanol residue (10g.) was subjected to silica gel column chromatography using hexane-ethyl-acetate : acetic acid (5:1:0.0085) as eluent. eighty fractions (25 ml each) collected, were examined by TLC. Fractions with similar chromatographic pattern were combined as shown in Table 2.

Table 2. Combined fractions from the Methanol residue (10 g)

Fractions	Number of elutes	Weight (g)
M 01	1 – 5	0.035
M 02	6 – 12	0.136
M 03	13 – 17	0.327
M 04	18 – 22	0.963
M 05	23 – 28	0.847
M 06	29 – 35	0.963
M 07	36 – 42	0.465
M 08	43 – 50	0.378
M 09	methanol elute	5.886

Fraction M03 was shown by TLC to contain interesting spots. It was separated by gel filtration, using chloroform-methanol (1:1) as an eluent. Fifty fractions (10 ml each) collected, were examined and combined accordingly with the information obtained the checked TLC as shown in table 3.

Table 3. Combined fractions from the M03 (0.327 g)

Fractions	Number of elutes	Weight (mg)
M 10	1 – 4	12
M 11	5 – 8	9
M 12	9 – 14	15
M 13	15 – 21	7
M 14	22 – 33	21
M 15	34 – 42	13
M 16	43 – 50	38
M 17	methanol elute	212

Isolation of Compound M

Fraction M13 containing pure anthraquinone spot, was crystallized in acetone to yield yellow thin needle (6 mg), designed as compound M.

The isolation of the crude methanol residue of the roots of *Morinda angustifolia* Roxb. were shown in scheme 1.

Fractionation of Methanol residue of the roots of *Morinda angustifolia* Roxb. (Scheme 2)

The crude methanol residue (40 g) was dissolved in aqueous methanol and partitioned with hexane, chloroform and methanol to give hexane extract (9.35 g), chloroform extract (6.48 g) and methanol extract (24.17 g) respectively.

The hexane extract was subjected to silica gel column chromatography, using hexane-chloroform (1:1) as an eluent. One hundred and twenty fractions (15 ml each) collected, were examined and combined accordingly with the information obtained from the checked TLC as shown in table 4.

Table 4 Combined fractions from the Hexane extract (9.35 g)

Fractions	Number of elutes	Weight (g)
H 01	1 – 9	0.047
H 02	10 - 21	0.938
H 03	22 – 47	1.335
H 04	48 – 64	1.546
H 05	65 – 69	1.483
H 06	70 – 75	0.011
H 07	76 - 120	0.742
H 08	methanol elute	3.248

Isolation of Compound H-2 and H-3

Fraction H 05 was shown by TLC to contain several spots of anthaquinone and one spot of steroid. The portion was subject to silica gel column chromatography using chloroform as an eluent. Two hundred and fifty fractions (5 ml each) collected, were examined and combined accordingly with the information obtained from the checked TLC as shown in Table 5.

Table 5 Combined fractions from the H 05 (1.483 g)

Fractions	Number of elutes	Weight (mg)
H 09	1 – 8	8
H 10	9 – 16	39
H 11	17 – 18	6
H 12	19 – 43	185
H 13	44 –56	246
H 14	57 –93	173

Table 5 Combined fractions from the H 05 (continued)

Fractions	Number of elutes	Weight (mg)
H 15	94 –125	32
H 16	126 –177	24
H 17	178 – 191	12
H 18	192 – 204	17
H 19	205 – 250	28
H 20	methanol elute	713

Isolation of Compound H-2

Fraction H11 was shown by TLC to contain a spot of steroid. It was crystallized in chloroform as colorless needle (4 mg), designed as H-2.

Isolation of Compound H-3

Fraction H15 was shown by TLC to contain a spot of anthraquinone and traces of impurity. It was further separated by gel filtration, using chloroform as an eluent. Eighty fractions (5 ml each) collected, were examined and combined accordingly with the information obtained from the checked TLC as shown in Table 6.

Table 6 Combined fractions from the H15 (32 mg)

Fractions	Number of elutes	Weight (mg)
H 21	1 – 8	3
H 22	9 – 14	1
H 23	15 – 33	4
H 24	34 – 39	2
H 25	40 – 55	6
H 26	56 – 64	4
H 27	65 - 80	3
H 28	methanol elute	9

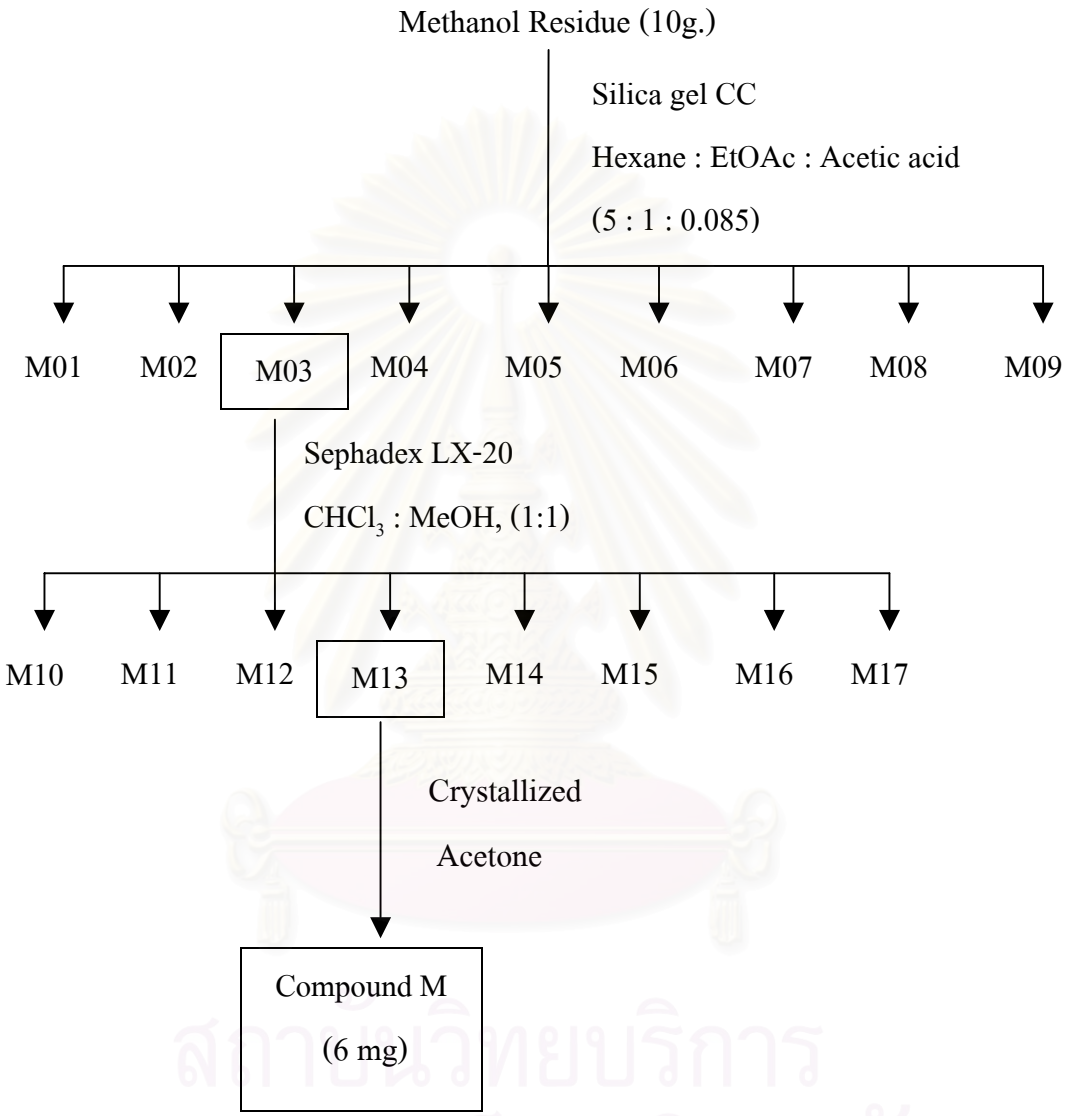
Fraction H25, containing pure anthraquinone spot was crystallized in acetone to yield yellow-orange needle (5 mg), designed as H-3.

Isolation of Compound H-4

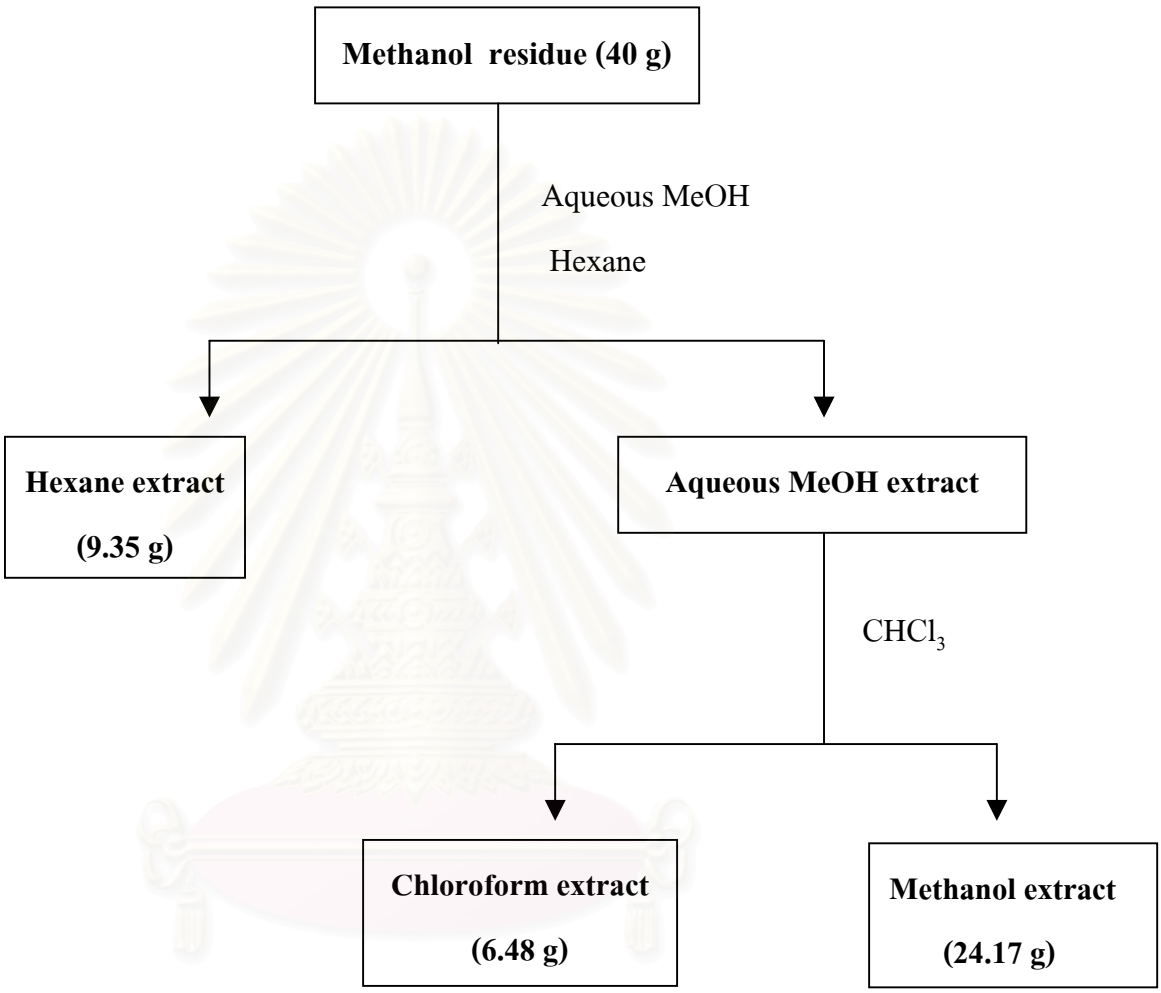
Fraction H06 was shown by TLC to contain a spot of anthraquinone. It was crystallized as orange-red thin needle (3 mg) in acetone, designed as H-4.

The Isolation of the Hexane Extract of the roots of *Morinda angustifolia* Roxb. were shown in scheme 2,3.

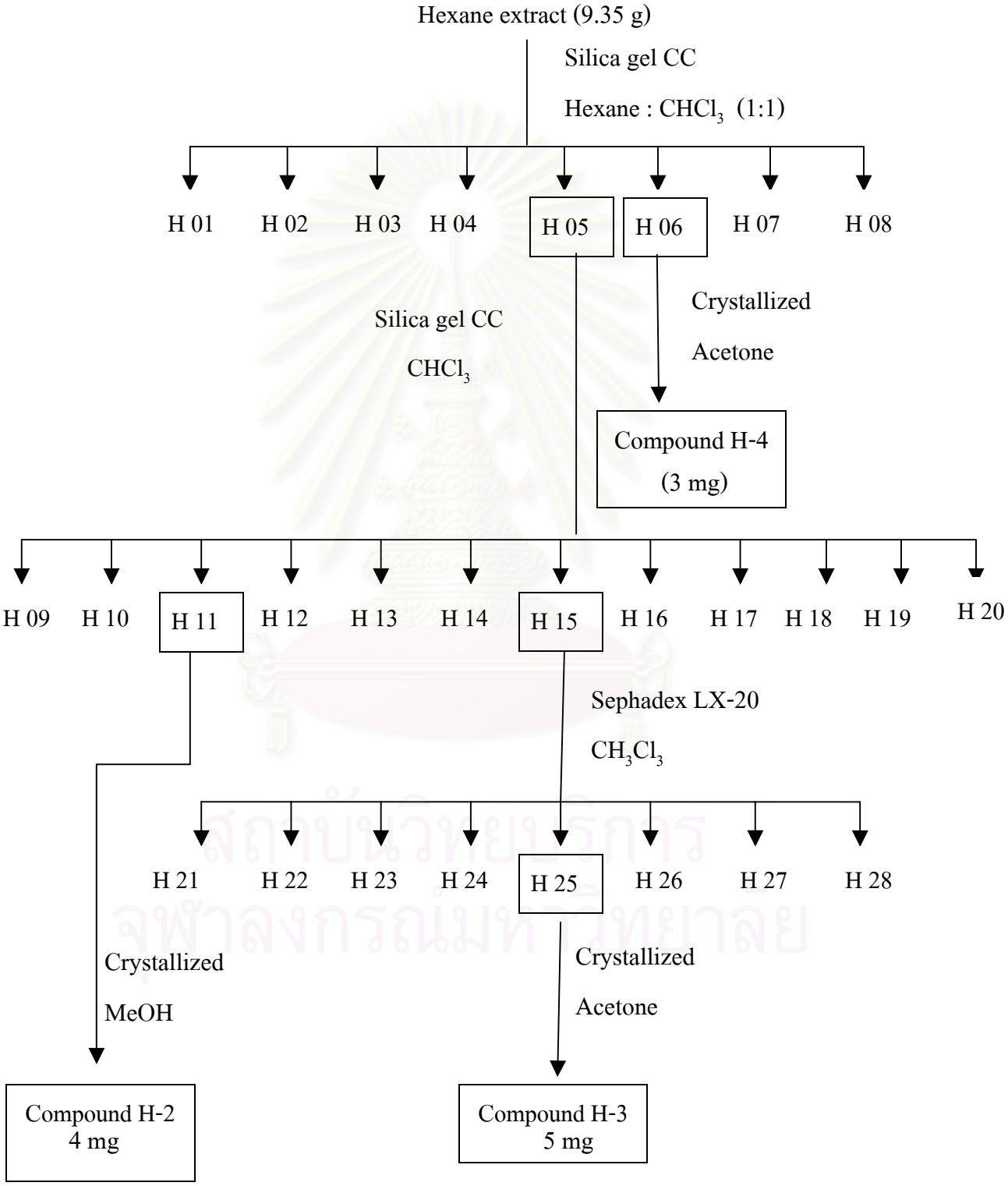
Scheme 1. Isolation of Methanol residue of the roots of *Morinda angustifolia* Roxb.



Scheme 2. Fractionation of Methanol residue of the roots of *Morinda angustifolia* Roxb.



Scheme 3. Isolation of Hexane extract of the roots of *Morinda Angustifolia* Roxb.



5. Physical and Spectral data of isolated Compounds

5.1 Compound M

Compound M was recrystallized in acetone as yellow needles.

mp : 194-195^{o}C

EIMS : *m/z* (% relation intensity); Figure 4.

282 (8), 254 (M^{+}100), 234 (89), 225 (40), 208 (24), 197 (28), 185 (41), 180 (93), 171 (29), 152 (38), 139 (19), 102 (13), 77 (10)

UV : λ_{max} nm (log ε), in methanol; Figure 2.

247(1.01), 280(0.96) and 377 (0.16)

IR : ν_{max} (KBr) disc cm^{-1} ; Figure 3.

3436,3067, 2956, 1646, 1565, 1445, 1388, 1343, 1260, 1219, 1062, 979, 768, 719 and 620

^{1}H NMR : δ (ppm), 500 MHz, in $CDCl_3$, Table 7; Figure 5a-5b.

4.11 (3H, *s*), 7.67 (1H, *s*), 7.76 (1H, *td*, *J* = 7.6, 1.4 Hz), 7.82 (1H, *td*, *J* = 7.6, 1.5 Hz), 8.24 (1H, *dd*, *J*=7.63, 1.5 Hz), 8.28 (1H, *dd*, *J*=7.6, 1.4 Hz), 10.46 (1H, *b*) and 12.26 (1H, *s*)

^{13}C NMR : δ (ppm), 125 MHz, in $CDCl_3$, Table 7; Figure 6a-6b.

64.74 (*s*), 113.14 (*s*), 117.68 (*s*), 118.66 (*s*), 127.13 (*s*), 127.41 (s), 132.51 (*s*), 133.67 (*s*), 134.84 (*s*), 134.89 (*s*), 141.67 (*s*), 166.66 (*s*), 180.20 (*s*), 181.96 (*s*) and195.48 (*s*)

5.2 Compound H-2

Compound H-2 was recrystallized in methanol as colourless needles.

Mp : 144-145

EIMS : *m/z* (% relation intensity); Figure 12.

412 (M^+, 15), 255 (32), 213 (64), 199 (36), 173 (41), 171 (43), 161 (50), 159 (83), 145 (93), 133 (65), 107 (62), 106 (100), 91 (75), 81 (61) and 79 (55)

^{1}H NMR : δ (ppm), 500 MHz, in $CDCl_3$; Figure 13.
5.17 (1H, *dd*, *J* = 15.0, 8.4 Hz), 5.0 (1H, *dd*, *J* = 15.0, 8.4 Hz), 5.32 (*m*), 3.49 (*m*), 0.68 (*s*), 0.99 (*s*).

5.3 Compound H-3

Compound H-3 was recrystallized in acetone as orange needles.

mp : 223-224 °C

EIMS : *m/z* (% relation intensity); Figure 16.
254 (15), 226 (26), 197 (58), 169 (20), 152 (21), 141 (25), 139 (16), 121 (9), 115 (31)

UV : λ_{max} nm (log ε), in methanol; Figure 14.
217(0.37), 268(0.35) 351, (1.02), 410(0.01) and 498(0.01)

IR : ν_{max} (KBr) disc cm^{-1} ; Figure 15.
3458, 3091, 2929, 1628, 1603, 1597, 1452, 1381, 1357, 1285, 1261, 1255, 1096, 1033, 804, 733, 421

^{1}H NMR : δ (ppm), 500 MHz, in DMSO-*d6*; Table 8; Figure 17a-17b.
2.29 (3H, *bs*), 7.24 (1H, *dd*, *J*=8.5, Hz), 7.47 (1H, *br d*, *J*= 2.7 Hz), 8.12 (1H, *dd*, *J*=8.5 Hz)

^{13}C NMR : δ (ppm), 125 MHz, in $CDCl_3$, Table 8; Figure 18.
15.66 (*s*), 112.53 (*s*), 114.72 (*s*), 118.58 (*s*), 121.43 (*s*), 124.44 (*s*), 129.80 (*s*), 131.13 (*s*), 134.18 (*s*), 135.59 (*s*), 136.86 (*s*), 159.94 (*s*), 163.89 (*s*), 181.80 (*s*), 187.61 (*s*)

5.4 Compound H-4

Compound H-4 was recrystallized in acetone.

mp : 248-249^{o}C

EIMS : *m/z* (% relation intensity); Figure 25.

270(M^{+},100), 242 (44), 214 (12), 196 (8), 185 (7), 139 (11), 135(22), 129 (6), 115 (3)

UV : λ_{max} nm (log ε), in methanol; Figure 23.

229 (0.82), 257 (0.81), 298 (0.36), 444 (0.29), 528 (0.06)

IR : ν_{max} (KBr) disc cm^{-1} ; Figure 24.

3847, 3091, 2929, 1628, 1603, 1452, 1381, 1358, 1286, 1255, 1016, 802, 733, 421

^{1}H NMR : δ (ppm), 500 MHz, in DMSO-*d6*; Table 9; Figure 26a-26b.

2.30 (3H, *br s*), 7.20 (1H, *d*, *J*=8.2 Hz), 7.68 (2H, *s*), 7.72 (1H, d, *J*=8.2 Hz), 3.30 (1H, *m*)

^{13}C NMR : δ (ppm), 125 MHz, in $CDCl_3$, Table 9; Figure 27.

15.81 (*s*), 115.08 (*s*), 116.18 (*s*), 118.56 (*s*), 120.65 (*s*), 121.71 (*s*), 122.76 (*s*), 130.94 (*s*), 134.93 (*s*), 136.86 (*s*), 151.32 (C), 154.28 (*s*), 160.26 (*s*), 186.72 (*s*), 188.05 (*s*)

CHAPTER IV

RESULTS AND DISCUSSION

In this phytochemical investigation, the constituents of the roots of *Morinda angustifolia* Roxb. was extensively studies. Purification of the hexane extract of the roots by column chromatography yield two compound (H-3 and H-4) and a mixture (H-2), whereas the other compound (M) was isolated from crude methanol residue. The identification and structure elucidation of these compounds were based on spectroscopic data (UV, IR, NMR and mass spectra) and also confirmed by comparison with those reported in the literature. They were proposed to be either anthraquinones or steroid, both of which are abundantly found in this plant family (Rubiaceae).

Identification and structure elucidation

1. Identification of Compound M

Compound M was crystallized as yellow needle in acetone, and gave purple color to ammonia vapour, suggesting the present of an anthraquinone nucleus. The EI Mass spectrum of this compound (Figure 4) showed a molecular ion peak at *m/z* 282, suggesting its molecular formula is $C_{16}H_{10}O_5$. This supported by number of carbons and the proton integration in NMR spectra. The IR spectrum of Compound M (Figure 3) exhibited a broad bond at 3436 cm^{-1} (O-H stretching) indicating the presence of hydroxyl groups in the molecule, and absorption bands at 1677 (C=O), 1646 (C=O) and 1259 (C=O) cm^{-1}, suggesting the presence two carbonyl ketone and one aldehyde groups in the molecule.

The ^{13}C-NMR spectrum of Compound M (Figures 6a-6b) showed the signals of sixteen carbon atoms. The DEPT-90 and DEPT-135 (Figures 7a-7b) experiments helped in differentiate the signals of one methyl carbons, five methine carbons and ten quaternary carbons.

The methyl carbon at δ 64.74 ppm was assigned as methoxy carbon at C-2 (2-OCH_3). Five methine carbons signals at δ 113.14, 127.13, 134.84, 133.67 and 127.41 ppm were assigned as C-4, C-5, C-6, C-7 and C-8, respectively. The quaternary carbons at δ 141.67, 166.60, 117.68, 118.66, 132.51, 180.20, 181.96 and 195.48 ppm were assigned as C-1, C-2, C-4a, C-9a, C-10a, C-9, C-10 and aldehyde carbon at C-3 (3-CHO), respectively.

The ^{1}H-NMR spectrum of Compound M (Figures 5a-5b) displayed the signals of methoxy protons at δ 4.11 (3H, *s*) ppm , one aldehyde proton at δ 10.46 (1H, *s*) ppm, one cheated hydroxy proton at δ 12.26 (1H, *s*) ppm, which were assigned as 1-OH, 2-OCH_3 and 3-CHO, respectively. The five aromatic protons.at δ 7.67 (1H, *s*), 7.77 (1H, *td*, *J* = 7.6, 1.4), 7.82 (1H, *td*, *J* = 7.6, 1.5), 8.24 (1H, *dd*, *J* = 7.6, 1.5) and 8.28 (1H, *dd*, *J* = 7.6, 1.4) ppm were assigned as H-4, H-6, H-7, H-5 and H-8, respectively.

The ^{1}H-^{1}H COSY experiment (Figures 8a-8b) showed cross peak of aromatic protons at δ 8.28 ppm (*dd*, *J* = 7.6, 1.4 Hz) and 7.82 ppm (*dd*, *J* = 7.6, 1.4 Hz), indicated the *ortho* coupling of aromatic protons assigned to protons at C-7 and C-8. The cross peak of aromatic protons at δ 8.24 ppm (*dd*, *J* = 7.6, 1.5 Hz) and 7.76 ppm (*td*, *J* = 7.6, 1.4 Hz), indicated the *ortho* coupling of aromatic protons assigned to protons at C-5 and C-6, respectively.

The HMBC experiment (Figures 11a-11e) was useful to confirm the whole structure of Compound M. The proton at δ 7.67 ppm (*s*) displayed three-bond correlation with C-2 (δ 166.66 ppm), C-9a (δ 118.66 ppm) and C-10 (δ 181.96 ppm), two-bond correlation with, C-3 (δ 134.89 ppm) and C-4a (δ 117.68 ppm), confirming its position at C-4. The aldehyde proton, at δ 10.46 ppm displayed three-bond correlation with C-2 (δ 116.66 ppm) and C-4 (δ 113.14 ppm) confirming its substitution at C-3.

The methyl protons at δ 4.11 (*s*) ppm displayed three bond correlation with C-2 (δ 116.66 ppm), confirming its substution at C-2 (δ 64.74 ppm). The chelated hydroxy proton at δ 12.26 ppm displayed three bond correlation with C-2 (δ 116.66 ppm) and C-9a (δ 118.66 ppm), confirming its substitution at C-1.

Therefore it could be concluded that Compound M is 1-hydroxy-2-methoxy-3-formyl anthraquinone. The complete carbon and proton assignment of Compound M together with HMBC, COSY and NOESY data was shown in table 7 and the HMBC correlation was shown below.

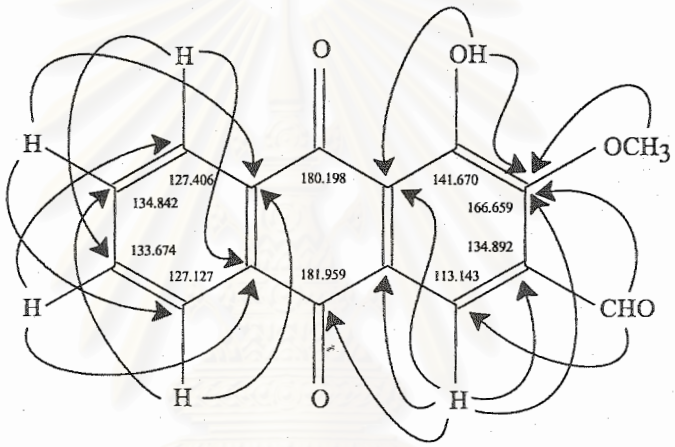


Table 7. The ^{1}H-NMR, ^{13}C-NMR, HMBC, COSY and NOESY data of Compound M

Position	δ C (ppm)	δ H (ppm)	HMBC correlations	COSY	NOESY
1	141.67	-			
2	166.66	-			
3	134.89	-			
4	113.14	7.67 (*s*)	C-4a, C-9a, C-10, C-2, C-3		2-CHO
5	127.18	8.27 (*dd*, *J*=7.6, 1.5 Hz)	C-7, C-8a, C-10	H-6	H-7
6	133.67	7.76 (*dt*, *J*=7.6, 1.4 Hz)	C-6, C-10a	H-5	
7	134.84	7.82 (*dt*, *J*=7.6, 1.5 Hz)	C-3, C-10a, C-9	H-8	H-5
8	127.40	8.28 (*dt*, *J*=7.6, 1.4 Hz)		H-7	
9	180.20				
10	181.96				
4a	117.68				
10a	132.51				
8a	134.89				
9a	118.66				
1-OH	-	12.26 (*s*)	C-2, C-9a		HO
2-OCH_3	64.74	4.11 (*s*)	C-2		HO
3-CHO	195.48	10.46 (*s*)	C-2, C-4		3-OCH_3 1-OH, H-4

2. Identification of compound H-2

Compound H-2 was obtained as colorless needle. It gave purple color upon spraying with 10% sulfuric acid in 95 % ethanol and heated. Liberman Burchard Test of this compound gave positive green color, suggesting the presence of a steroidal skeleton.

The EI Mass spectrum of Compound H-2(Figure 12) displayed a prominent molecular ion peak at *m/z* 412 and 414, suggesting its molecular formular $C_{29}H_{48}O$ and $C_{29}H_{50}O$, indicated the mixture of two compound. EIMS fragment peak at *m/z* 217 ([M-$C_{10}H_{21}$]) was also important in showing Compound H-2 as having a steroid skeleton (Rubinstein *et al*., 1976).

The ^{1}H-NMR spectrum of compound H-2 (Figure 13) displayed the signals at δ 5.12 ppm (1H, *dd*, *J* = 15.0, 8.4 Hz) and δ 5.00 ppm (1H, *dd*, *J* = 15.0, 8.4 Hz) assigned to H-23 and H-22 of the disubsituted double bond in the side chain of stigmasterol. Another vinylic proton appeared as a multiplet at δ 5.33 ppm, was assigned to H-6 of both *β*-sitosterol and stigmasterol, respectively. Another multiplet at δ 3.50 ppm was attributable to the proton germinal to the 3-OH group. The signal at δ 0.68-0.99 ppm represented the angular methyl group at H-18 and H-19, respectively. The integrated peak areas of H-6 and either H-22 or H-23 were in the ratio of 2 : 1. Therefore, it could be deduced that compound H-2 was a 1:1 mixture of *β*-sitosterol and stigmasterol.

3. Identification of compound H-3

Compound H-3 was crystallized as yellow needle in acetone, and gave purple color to ammonia vapour, suggesting the present of an anthraquinone nucleus. The EI Mass spectrum of this compound (Figure 16) showed a molecular ion peak at *m/z* 254, suggesting its molecular formula $C_{15}H_{10}O_4$. This supported by number of carbon and the proton integration in the NMR spectra. The IR spectrum (Figure 15)

revealed absorption bands at 1667 and 1636 cm^{-1}, suggesting the presence two carbonyl ketone groups.

The ^{13}C-NMR spectrum (Figure 8) of Compound H-3 exhibited the signals of fifteen carbon atoms. The DEPT-135 and DEPT-90 (Figure 19) experiment were performed to differentiate these 16 signals into those of one methyl carbon, six methine and nine quaternary carbons.

The methyl carbon at δ 15.66 ppm was assigned as methyl carbon at C-2 (2-CH_3). The methine carbons at δ 112.53, 118.58, 121.43, 129.80 and 136.83 ppm were assigned as C-5, C-4, C-7, C-8 and C-3 respectively. The quaternary carbons at δ 114.72, 124.44, 131.13, 134.18, 135.59, 159.94, 163.89, 187.61 and 181.80 ppm were assigned as C-9a, C-8a, C-4a, C-2, C-10a, C-1, C-6, C-9 and C-10, respectively.

The ^{1}H-NMR spectrum (Figures 17a-17b) showed signals of methyl protons at δ 2.30 (*s*) ppm and chelated hydroxy protons at δ 13.12 (*brs*) ppm which were assigned as methyl proton at C-2 and chelated hydroxy proton at C-1, respectively. Five aromatic protons at δ 7.24 (1H, *dd*, *J* = 8.5 Hz), δ 7.47 (1H, *d*, *J* = 2.7 Hz), δ 8.12 (1H, *dd*, *J* = 8.5 Hz), δ 7.65 (1H, *d*, *J* = 7.6 Hz), and δ 7.60 (1H, *d*, *J* = 7.6 Hz) were assigned as H-7, H-5, H-8, H-3 and H-4, respectively.

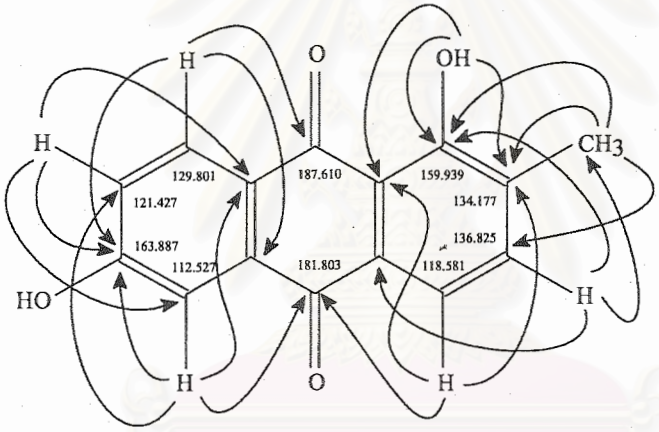
The ^{1}H-^{1}H COSY experiment was shown cross peak of protons at δ 8.12 (1H, *dd*, *J* = 8.5 Hz) and δ 7.23 (1H, *dd*, *J* = 8.5 Hz) indicated *ortho* coupling of aromatic protons assigned to be H-8 and H-7. The cross peak of protons at δ 7.24 (1H, *dd*, *J* = 8.5 Hz) and δ 7.47 (1H, *br d*, *J* = 2.7 Hz) indicated *ortho* coupling of aromatic protons assigned to be H-7 and H-5, respectively.

The HMBC experiment (Figures 22a-22g) was useful to confirm the whole structure of Compound M. The chelated hydroxy proton at δ 13.12 ppm (*br s*) displayed three-bond correlation with C-9a (δ 114.72 ppm) and two-bond correlation with C-1 (δ 159.94 ppm) confirming its substitution at C-1.

The methyl protons at δ 2.30 ppm (*s*) displayed two-bond correlation with C-9a (δ 134.18 ppm) and three-bond correlation with C-1 (δ 159.94 ppm) and C-3 (δ 136.83 ppm), confirming its substitution at C-2.

The protons at δ 7.47 (*d*, $J = 2.7$ Hz) displayed three-bond correlation with C-10 (δ 181.80 ppm) and two-bond correlation with C-6 (δ 163.89 ppm), confirming its position at C-5 and the hydroxyl substitution at C-6.

Therefore it could be concluded that Compound H-3 is Soranjidiol. The complete carbon and proton assignments of Compound H-3 together with HMBC and COSY correlation was shown in Table 8, and the HMBC was shown below.



Soranjidiol is a known anthraquinone which was first isolated from the Indian crude medicine name Soranji (the roots of *M. citrifolia* Linn. and *M. tinctoria* Roxb. (Thomson, 1971). It has been found in several species of *Morinda* such as the roots of *M. elliptica* Ridl. (Ali *et al.*, 2000 and Ismail *et al.*, 1997), the roots (Sittie *et al.*, 1999) and the stem (Adesogan, 1973) of *M. lucida* Benth. and the root bark of *M. tomentosa* Heyne ex Roth. (Rao and Ro, 1983).

This is the first report of complete ^{1}H and ^{13}C-NMR assignment of this compound.

Table 8. .The[1]H-NMR, [13]C-NMR, HMBC and COSY data of Compound H-3

Position	δ C (ppm)	δ H (ppm)	HMBC correlations	COSY
1	159.94	-	-	
2	134.18	-	-	
3	136.86	7.65 (*br d*, *J*=7.6 Hz)	C-4a, C-1, 2-CH_3	
4	118.58	7.60 (*d*, *J*=7.6 Hz)	C-2, C-10a, C-10	
5	112.53	7.47 (*br d*, *J*=2.7 Hz)	C-8a, C-7, C-10, C-6	H-7
6	163.89	-	-	
7	121.48	7.24 (*dd*, *J*=8.5,2.7Hz)	C-8a, C-5, C-6	H-5, H-8
8	129.80	8.12 (*dd*, *J*=8.5,0.6 Hz)	C-10a, C-6, C-9	H-7
9	187.61	-	-	
10	181.80	-	-	
4a	131.13	-	-	
10a	114.72	-	-	
8a	124.44	-	-	
9a	135.59	-	-	
1-OH	-	13.12	C-1, C-2, C-10a	
2-OCH_3	-	2.29	C-2, C-3, C-1	
6-OH	-	13.12	-	

Identification of compound H-4

Compound H-4 was crystallized as yellow needle in acetone. The EI Mass spectrum of this compound (Figure 25) showed a molecular ion peak at *m/z* 270, suggesting its molecular formula $C_{15}H_{10}O_5$. This supported by number of carbons and the proton integration in the NMR spectra. The IR spectrum (Figure 24) revealed absorption bands at 1628 and 1603 cm^{-1}, suggesting the presence two carbonyl ketone groups.

The ^{13}C-NMR spectrum (Figure 27) of Compound H-4 exhibited the signals of fifteen carbon atoms. The DEPT-135 and DEPT-90 (Figure 28a-28b) experiment were performed to differentiate these 15 signals into those of one methyl carbon, four methine carbons and ten quaternary carbons

The carbon at δ 15.81 ppm was assigned as methyl carbon at C-6 (6-CH_3). Four methine carbons at δ 136.59, 118.56, 120.65 and 121.71 ppm were assigned as C-3, C-4, C-7 and C-8, respectively. The ten quaternary carbons at δ 160.26, 134.93, 151.32, 154.28, 130.94, 122.76, 115.08, 116.18, 188.05 and 186.05 ppm were assigned as C-1, C-2, C-5, C-6, C-4a, C-8a, C-9s, C-10a, C-9 and C-10, respectively.

The ^{1}H-NMR spectrum (Figures 26a-26b) showed four signals of aromatic protons, chelated hydroxy proton at δ 13.30 ppm (*m*) and methyl proton at δ 2.30 (*s*).

The signals at δ 7.68 (2H,*s*) ppm (Figure 26b) intergrated for two protons, was assigned as H-3 and H-4, respectively. The aromatic proton at δ 7.20 (1H, *d*, *J* = 8.2 Hz) and δ 7.72 (1H, *dd*, *J* = 8.2 Hz) were assigned as H-7 and H-8, respectively. The proton at δ 13.30 ppm (*m*) was assigned as hydroxy proton at C-5, whereas the protons at δ 2.30 (*s*) was assigned as methyl proton at C-6.

The ^{1}H-^{1}H COSY experiments was shown cross peak of aromatic protons at δ 7.20 (1H, *d*, *J* = 8.2 Hz) and δ 7.72 (1H, *d*, *J* = 8.2 Hz), indicated *ortho* coupling of aromatic protons at H-7 and H-8.

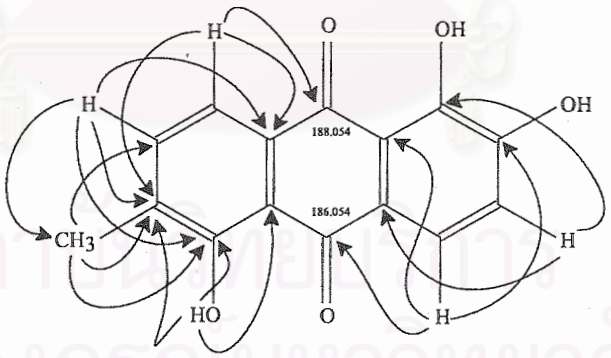
The HMBC experiment (Figures 32a-32g) was useful to confirm the whole structure. The chelated hydroxy proton at δ 13.30 ppm (*m*) displayed two-bond correlation with C-5 (δ 160.26 ppm) and three-bond correlation with C-6 (δ 134.93 ppm) and C-10a (δ 115.08 ppm) confirming its substitution at C-5.

The methyl protons at δ 2.30 ppm (*br s*) displayed two-bond correlation with C-6 (δ 134.93 ppm) and three-bond correlation with C-5 (δ 160.26 ppm) and C-7 (δ 136.86 ppm), confirming its position at C-6.

The proton at δ 7.72 ppm (*d*, J = 8.24 Hz) displayed three-bond correlation with C-10 (δ 186.05 ppm) and C-2 (δ 154.25 ppm), confirming its position at C-4 and the hydroxyl substitution at C-2.

The aromatic proton at δ 7.720 (*d*, J = 8.24 Hz) displayed three-bond correlation with C-4a (δ 122.759 ppm) and C-1 (δ 151.319 ppm), confirming its position at C-3 and the hydroxyl substitution at C-1.

Therefore it could be concluded that Compound H-4 is Morindone. The complete carbon and proton assignments of Compound H-4 together with HMBC COSY and NOESY data was shown in Table 9 and the HMBC was shown below.



Morindone is a known anthraquinone. It has been found in several species of *Morinda* such as cell suspension (Inouet *et al.*, 1981; Leistner, 1973 and Leistner, 1975), heartwood (Balakrishma *et al.*, 1961) and root bark (Balakrishma *et al.*, 1960) of *M. citrifolia* Linn., roots of *M. elliptica* (Ali *et al.*, 2000 and Ismail *et al.*, 1997), root

bark (Rao *et al*., 1997 and Murti *et al*., 1959) and heartwood (Eswaran *et al*., 1979) of *M.tinctoria* Roxb. and stem bark of *M. tomentosa* Heyne ex Roth. (Rao and Reedy, 1976).

This is the first report of complete ^{1}H and ^{13}C-NMR assignment of this compound.

Table 9. The ^{1}H-NMR, ^{13}C-NMR, HMBC, COSY and NOESY data of Compound H-4

Position	δ C (ppm)	δ H (ppm)	HMBC correlations	COSY	NOESY
1	160.26	-			
2	134.93	-			
3	136.59	7.68 (*s*)	C-1, 2-CH_3, C-2, C-4a		2-CH_3
4	118.56	7.68 (*s*)	C-9, C-2, C-4a		2-CH_3
5	151.32	-			
6	154.28	-			
7	120.65	7.20 (*d*, *J* = 8.2 Hz)	C-5	H-8	H-8
8	121.71	7.715 (*d*, *J* = 8.2 Hz)	C-6, C-10, C-10a	H-7	H-7
9	186.05	-			
10	188.05	-			
4a	130.94	-			
10a	116.18	-			
8a	122.76				
9a	115.08				
1-OH	-	13.30 (*br m*)	C-1, C-2, C-10a		
2-CH_3	15.81	2.30 (*br s*)	C-1, C-2, C-3		H-3, H-4
5-OH					
6-OH					

CHAPTER V

CONCLUSION AND RECOMMENDATION

The phytochemical investigation of the roots of *Morinda angustifolia* Roxb. revealed the presence of three pure compounds those of 1-hydroxy-2-methoxy–3–formyl anthraquinone, soranjidiol and morindone, and a mixture of stigmasterol and *β*-sitosterol which was found commonly in plants. The pharmacological study of the anthraquinone is one of the points strongly recommended. Further work should be done on the investigation of anthraquinones and some other compounds in the chloroform and methanol extracted.

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APPENDIX

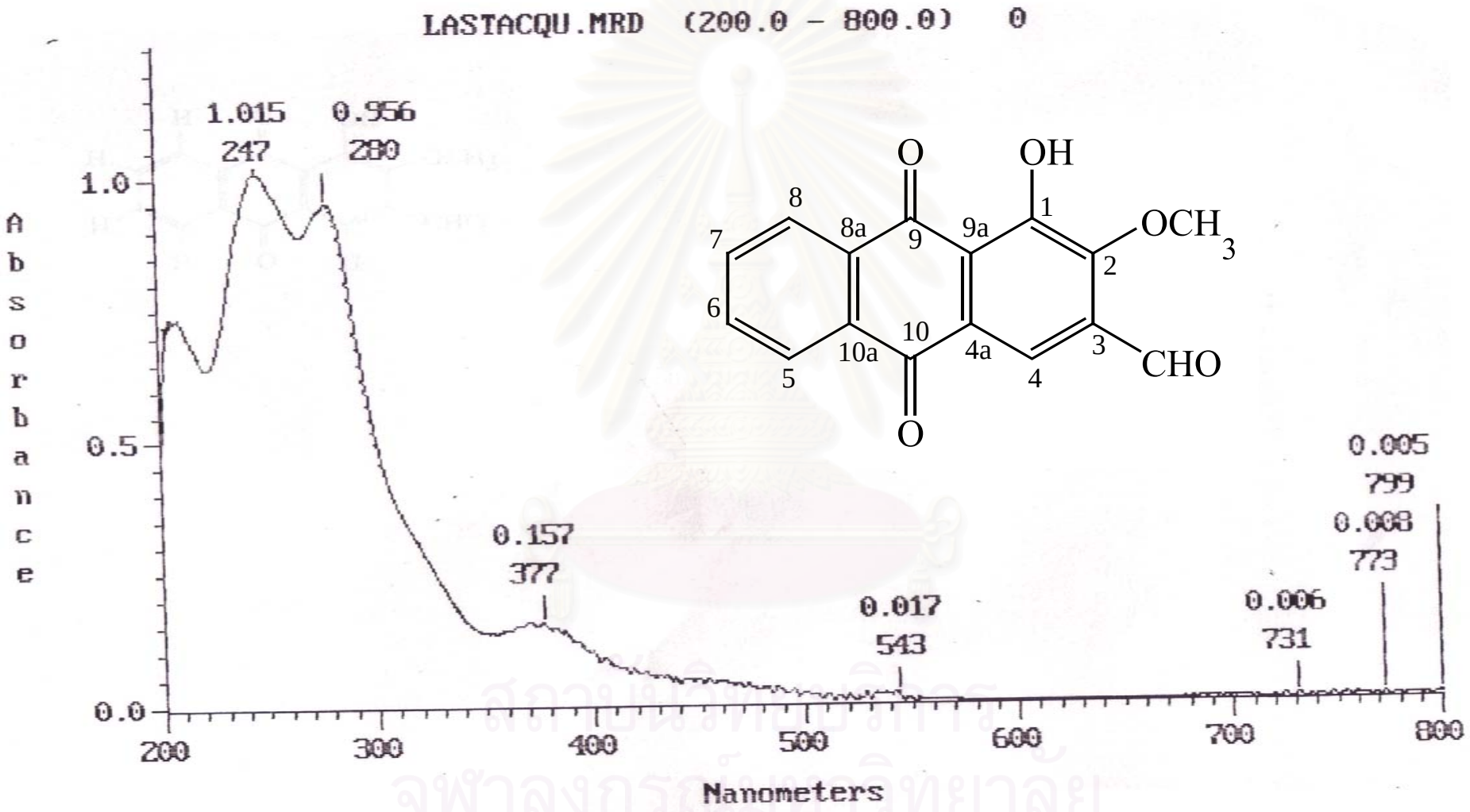


Figure 2. Ultraviolet absorption spectrum of Compound M (in MeOH)

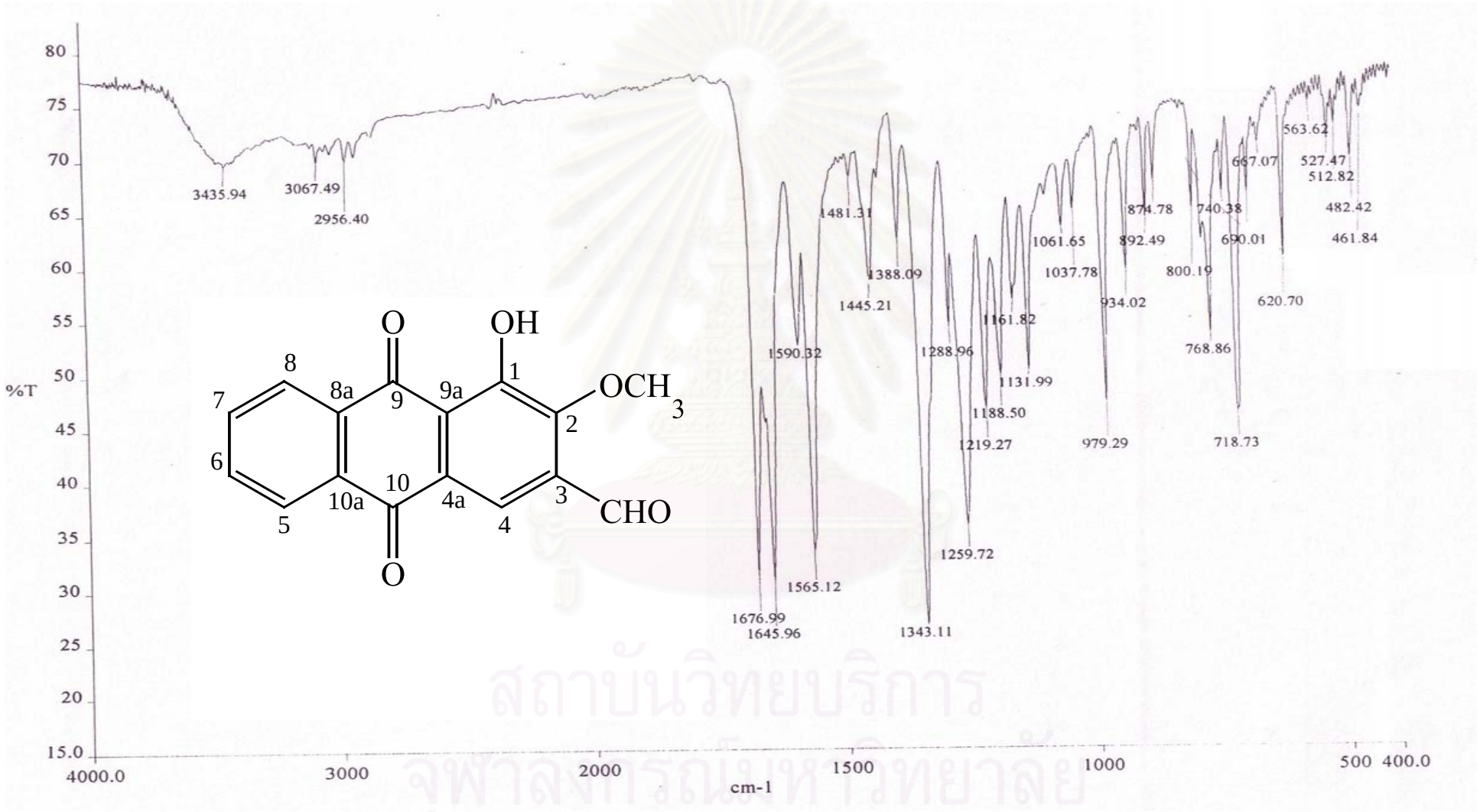


Figure 3. Infrared absorption spectrum of Compound M (KBr)

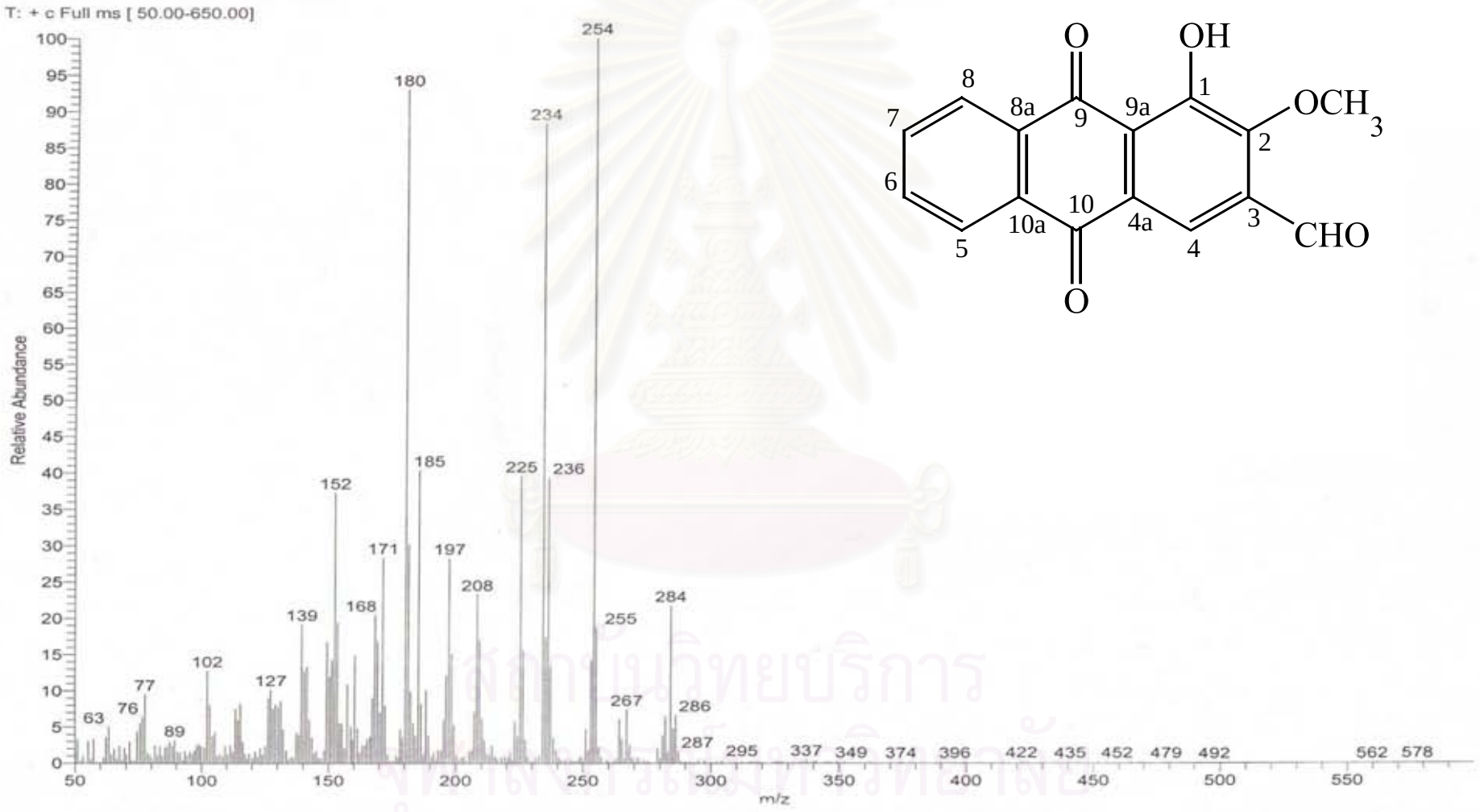


Figure 4. EI Mass spectrum of Compound M

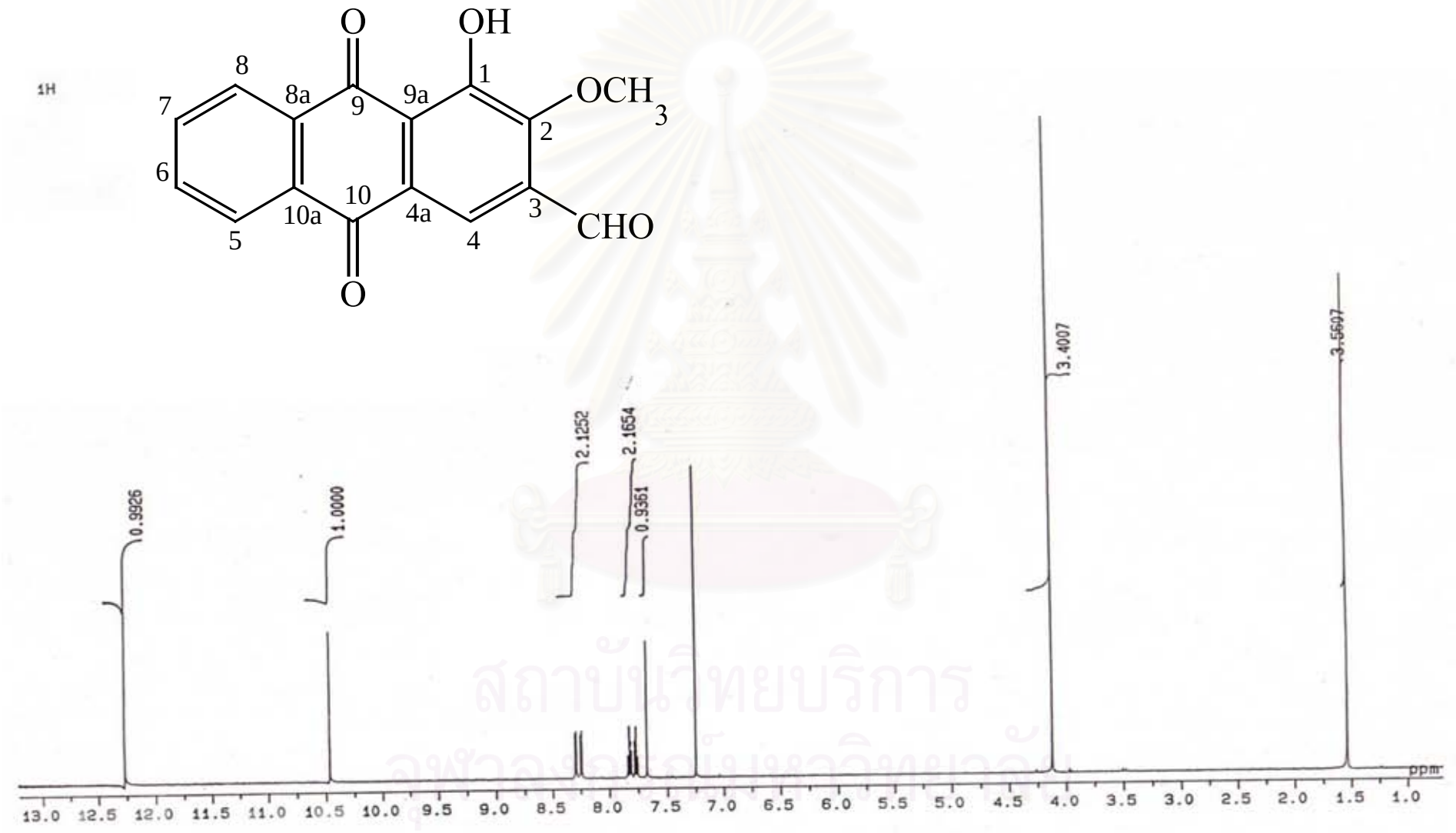


Figure 5a. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound M in $CDCl_3$

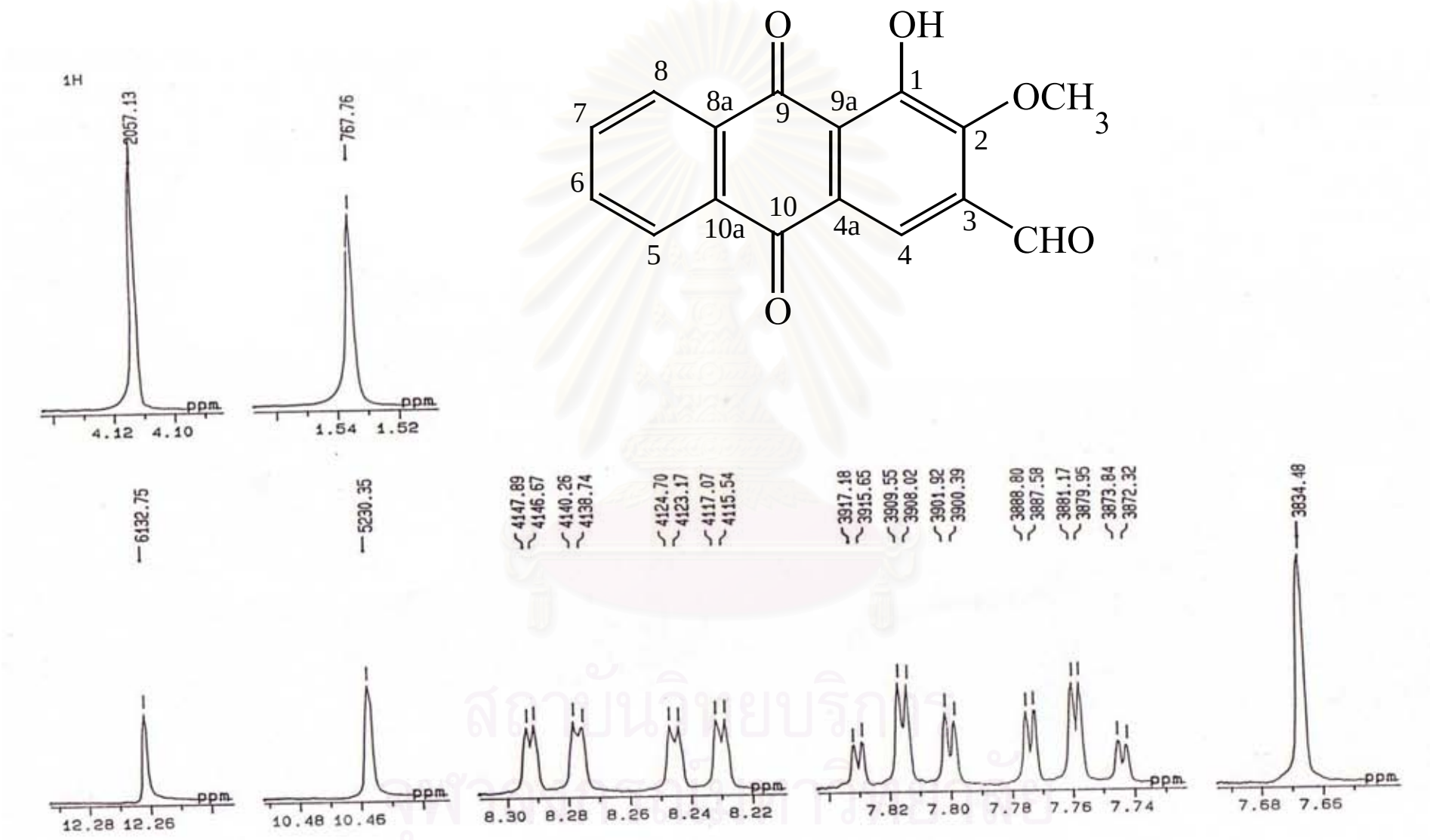


Figure 5b. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound M in $CDCl_3$ (expanded)

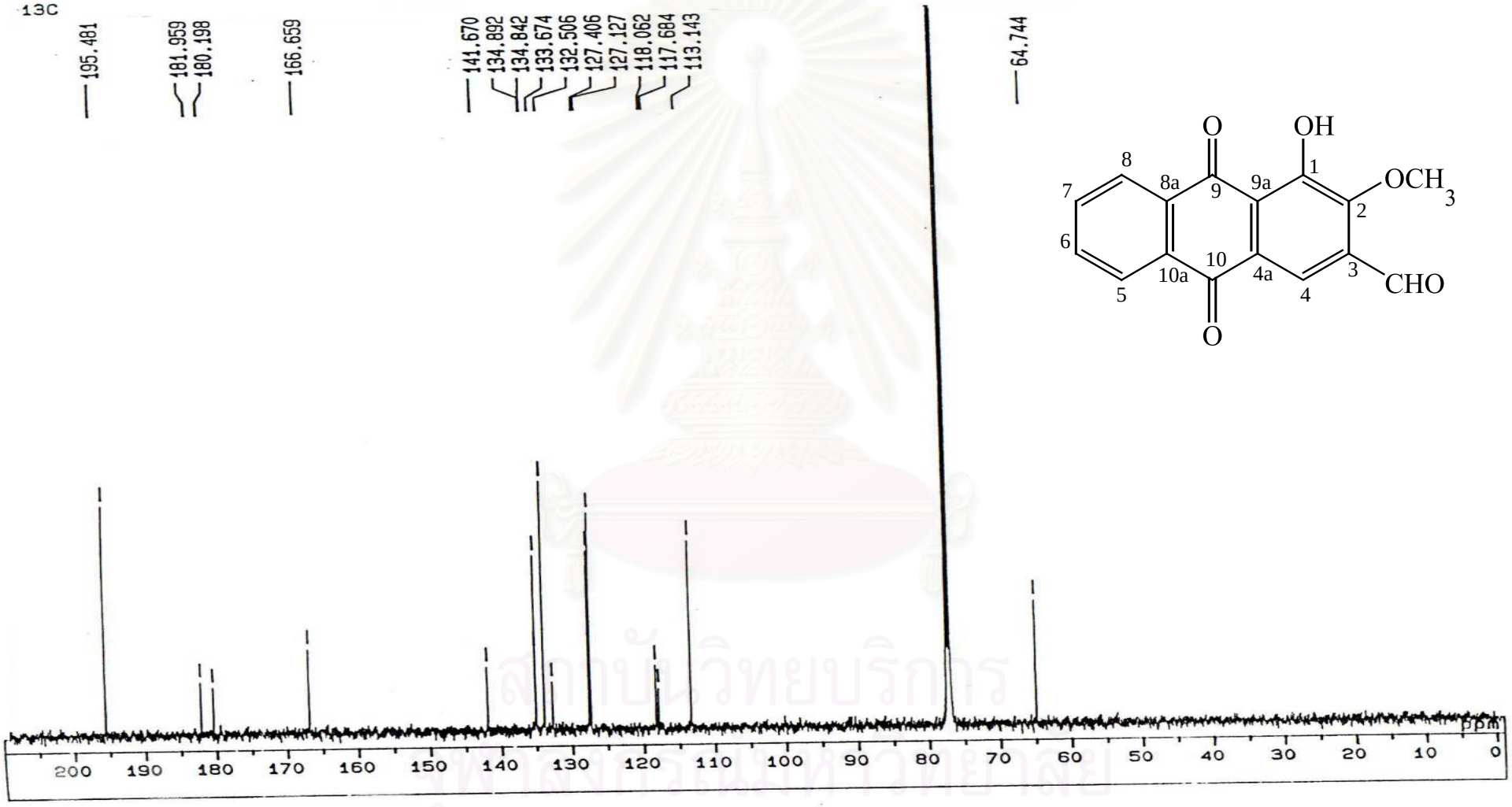


Figure 6a. The ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound M in $CDCl_3$

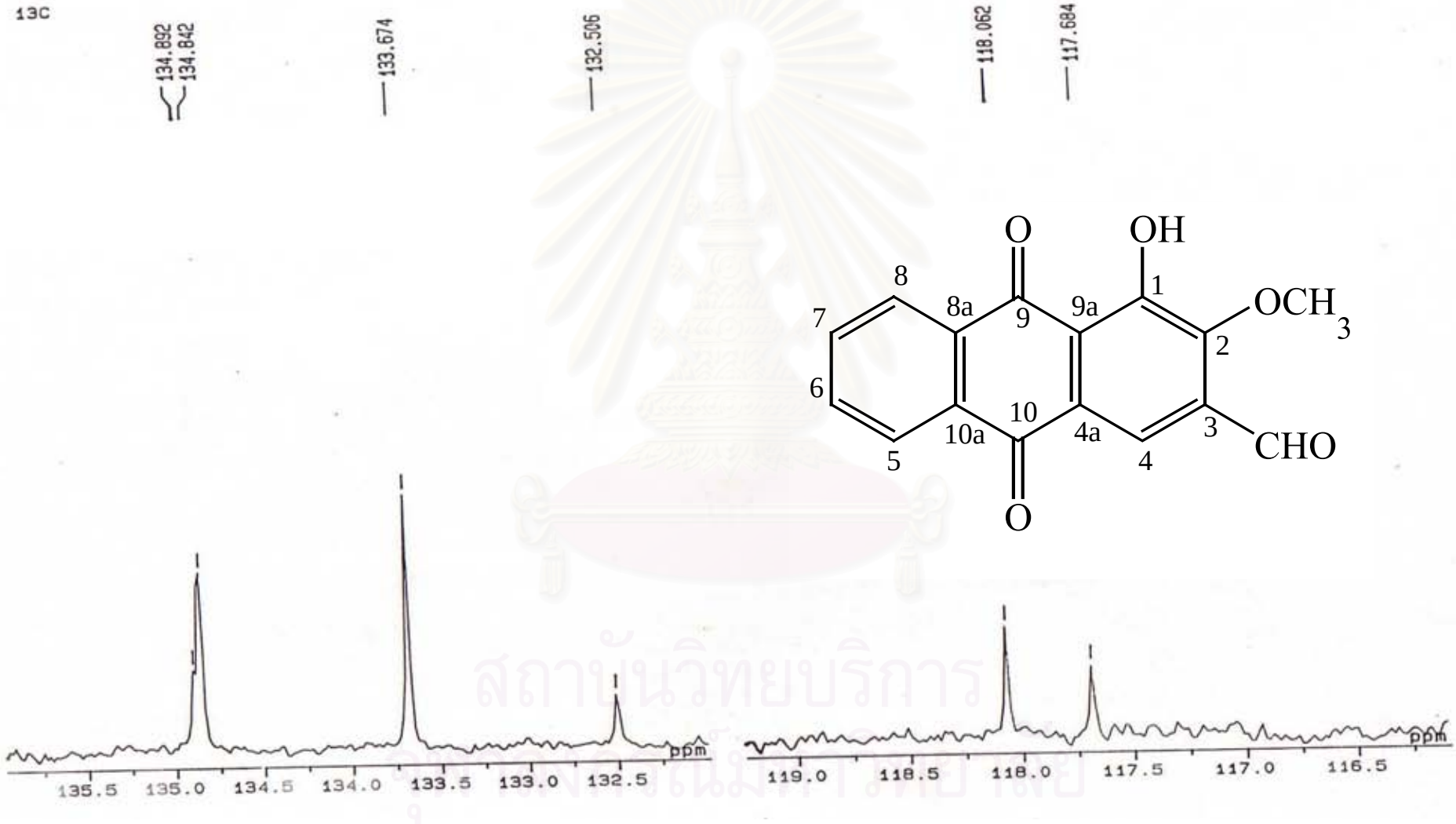


Figure 6b. The ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound M in $CDCl_3$ (expanded)

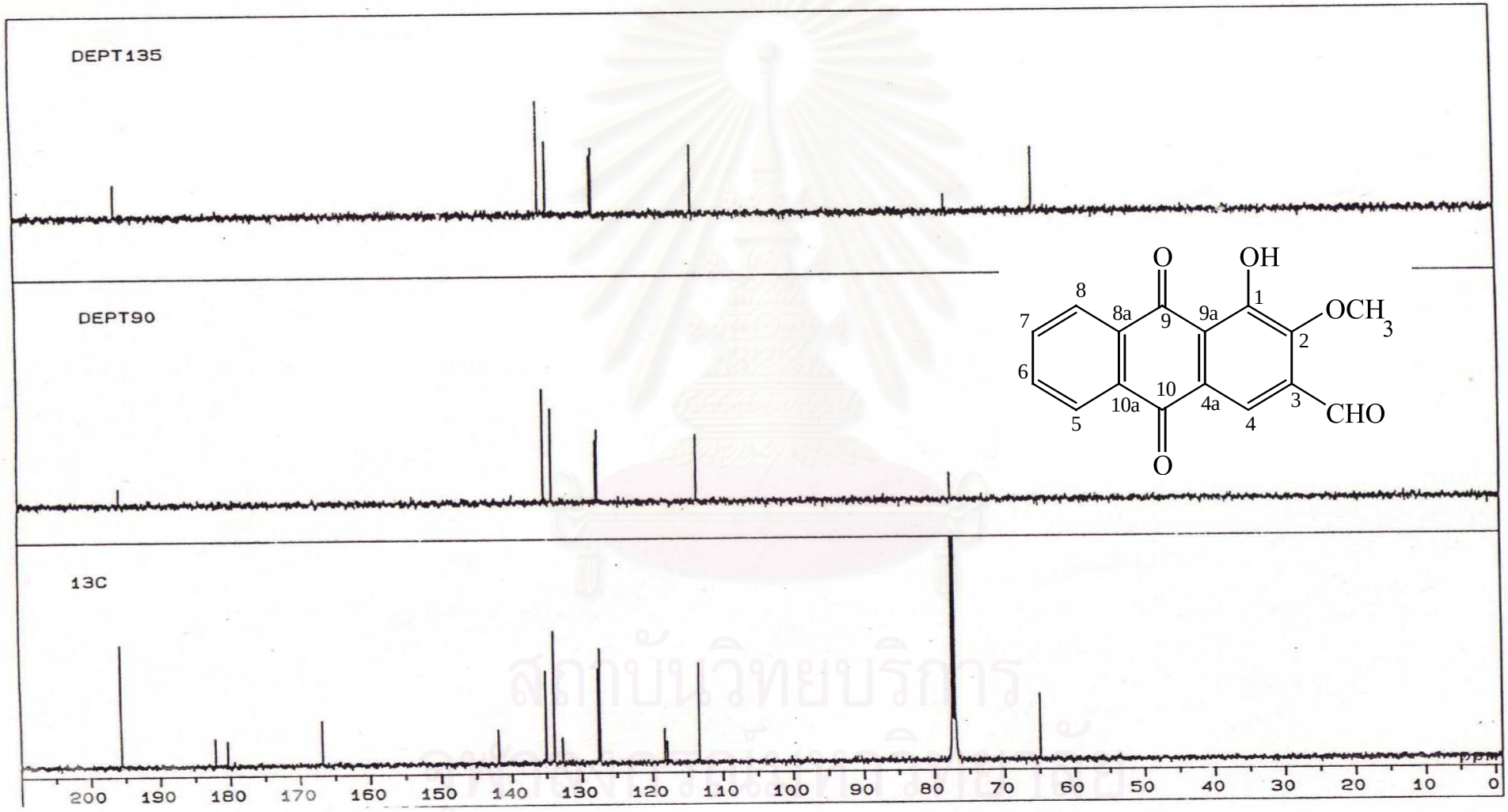


Figure 7a. The DEPT-135, 90 and ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound M in $CDCl_3$

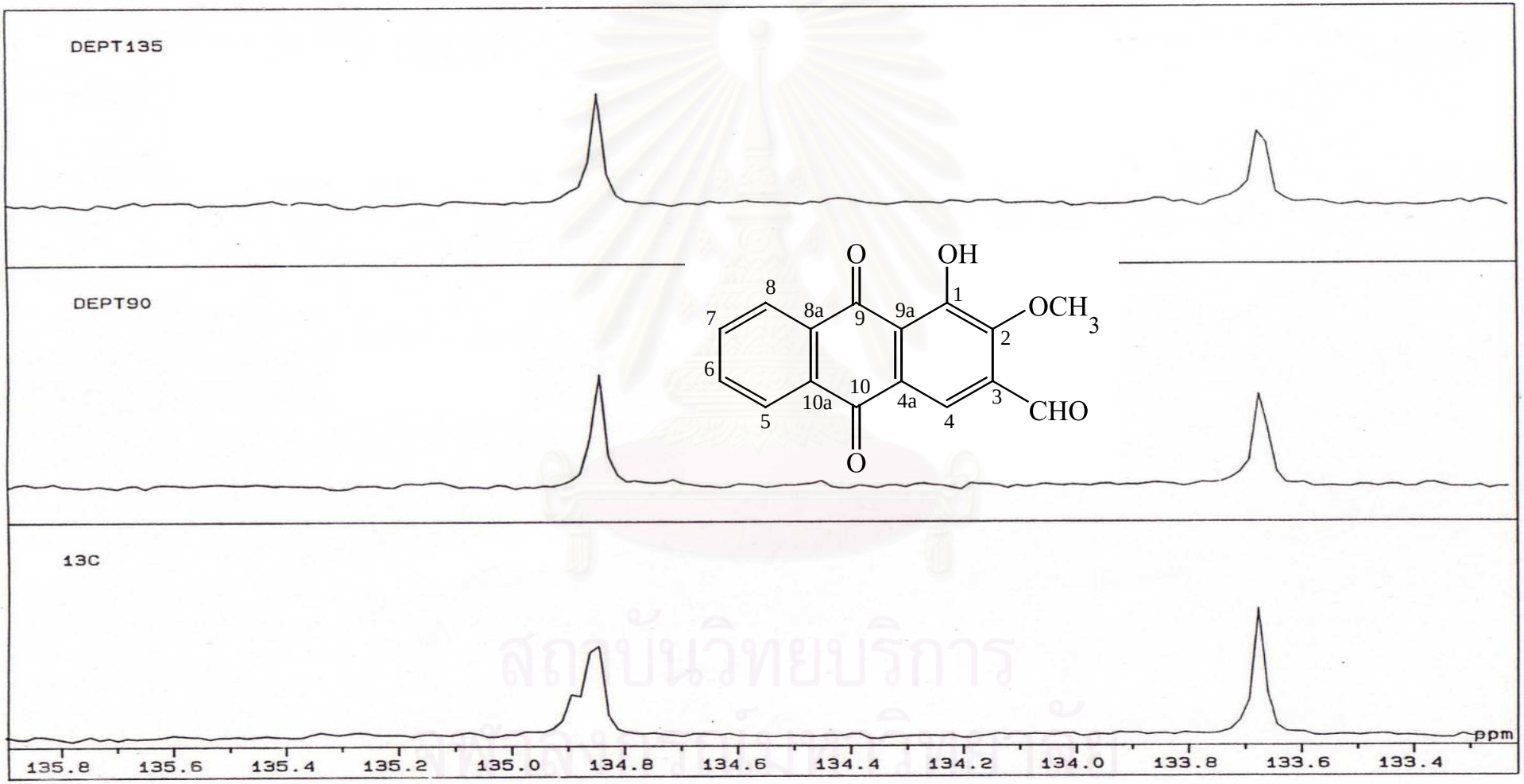
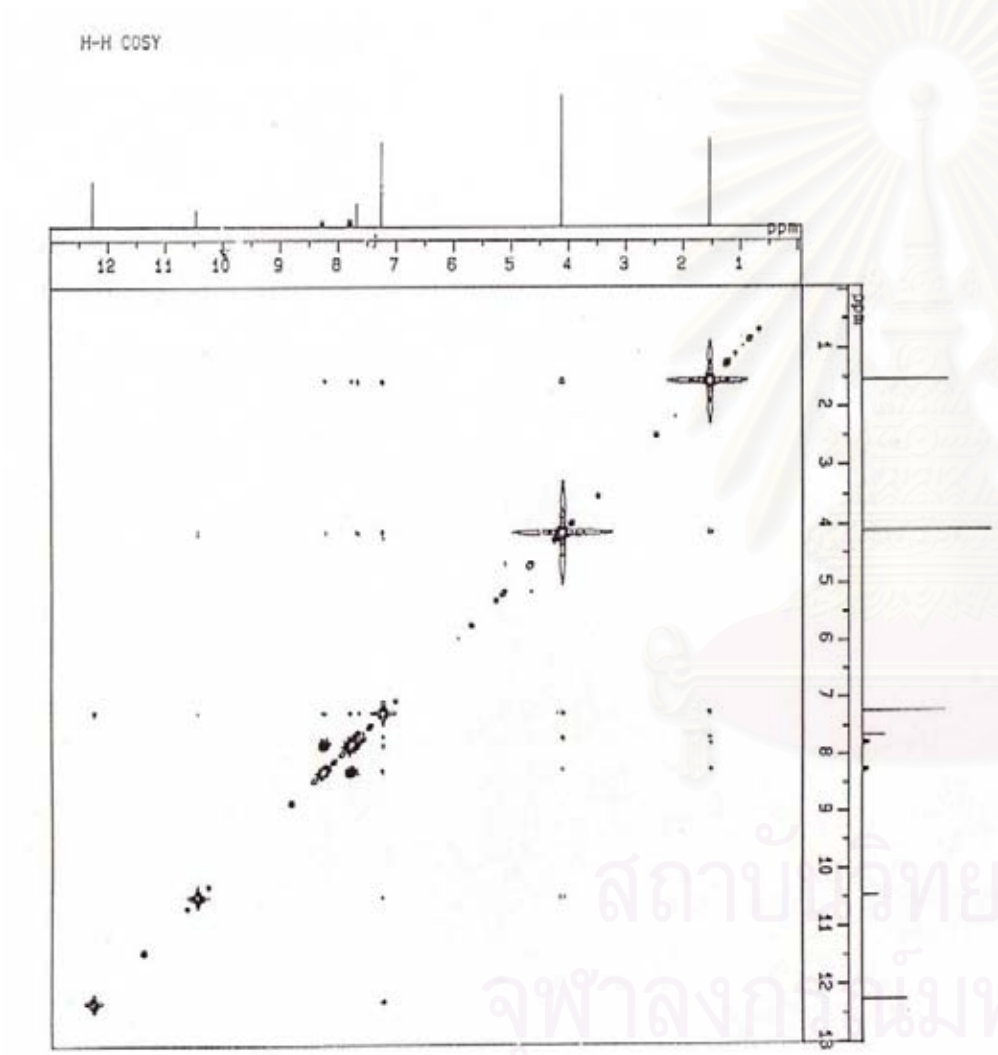


Figure 7b. The DEPT-135, 90 and ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound M in $CDCl_3$ (expanded)



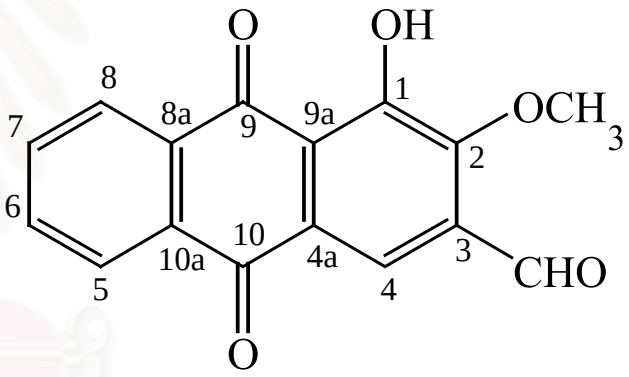
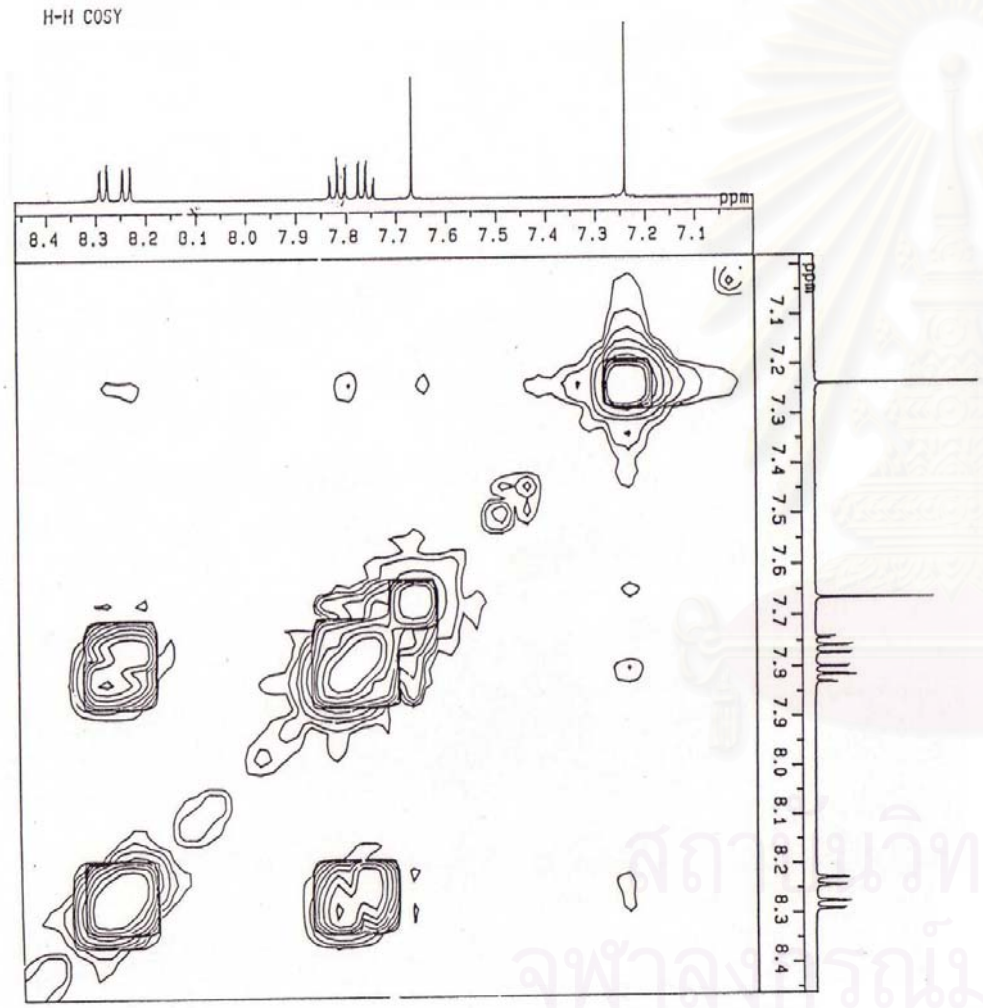


Figure 8a. The ^{1}H-^{1}H COSY spectrum of Compound M in $CDCl_3$



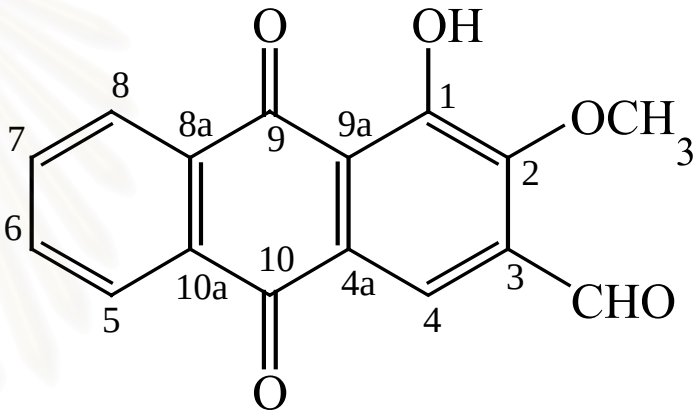
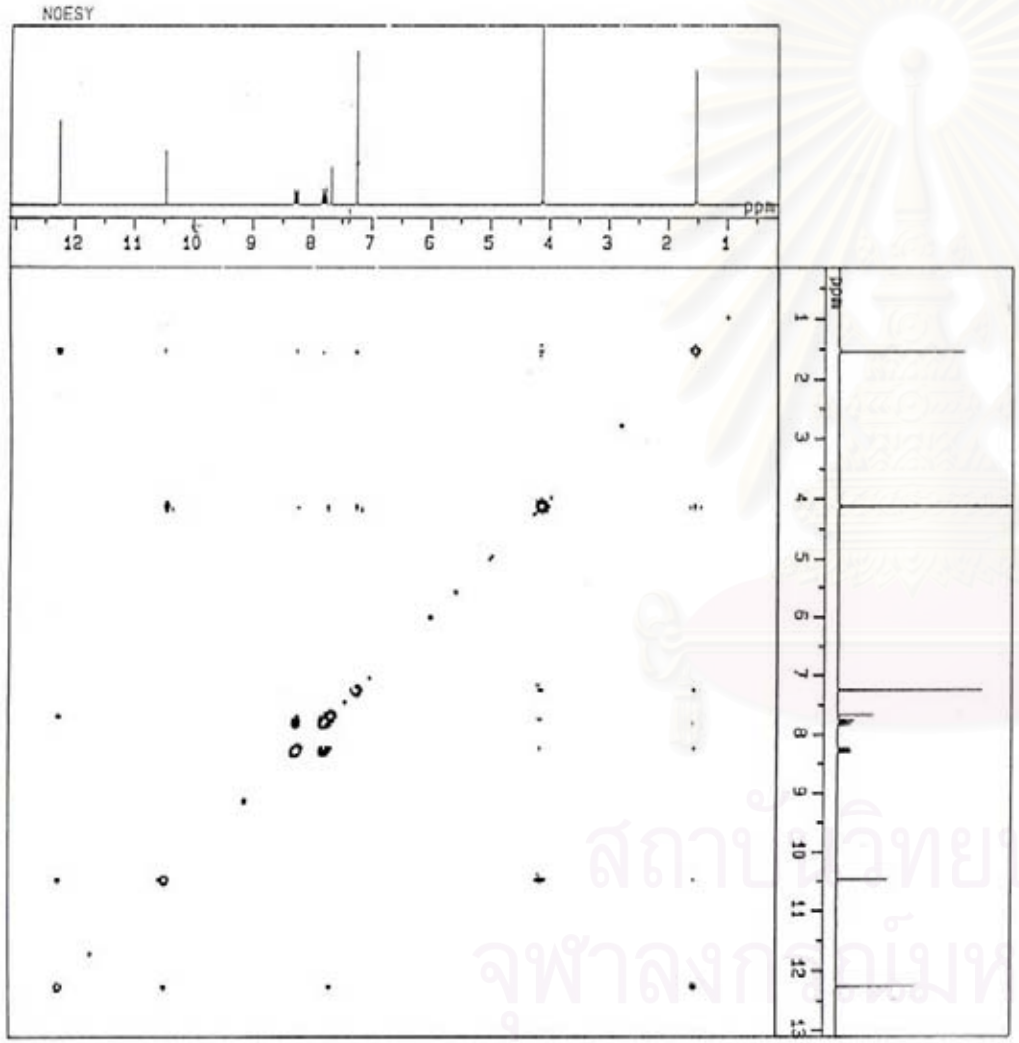


Figure 8b. The ^{1}H-^{1}H COSY spectrum of Compound M in $CDCl_3$ (expanded)



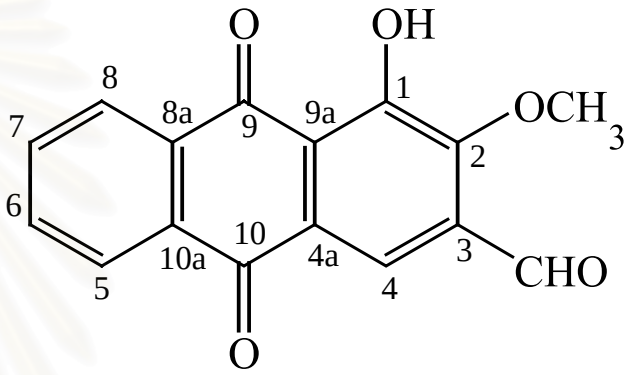
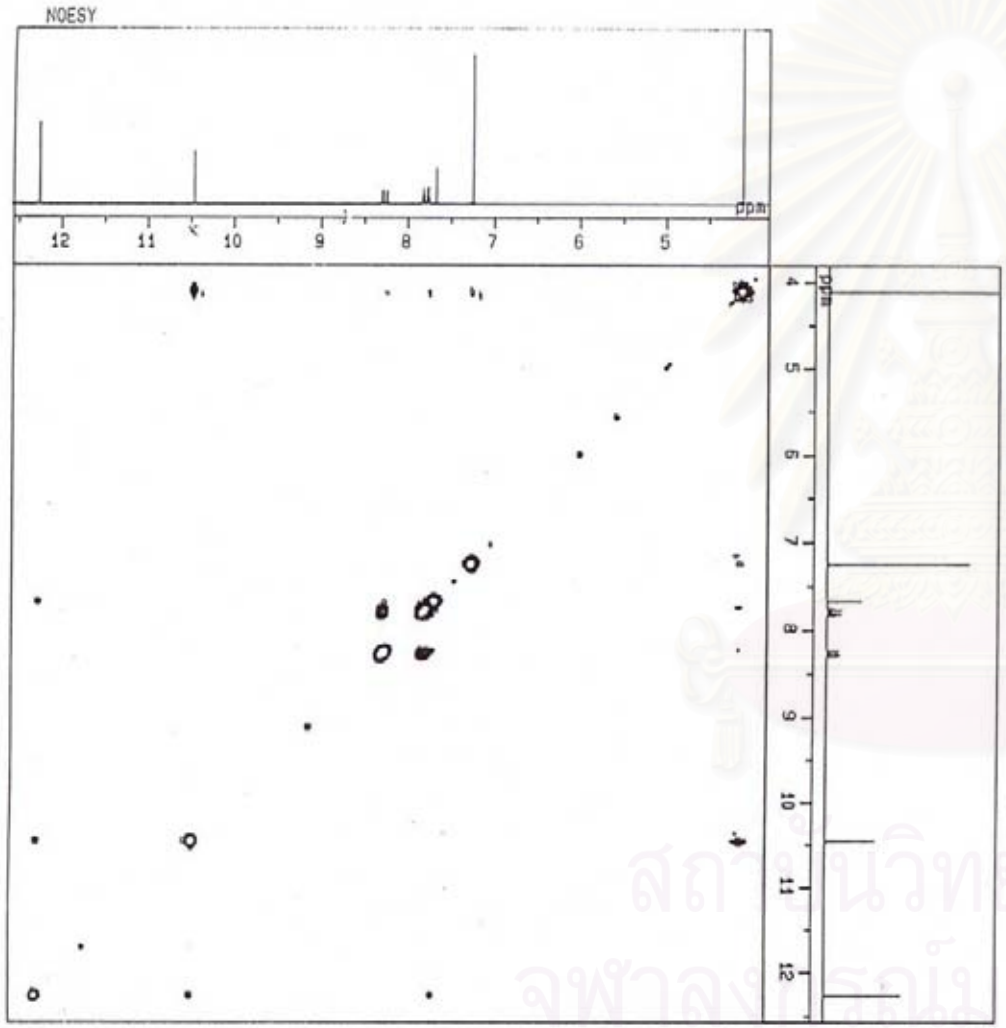


Figure 9a. The NOESY spectrum of Compound M in $CDCl_3$



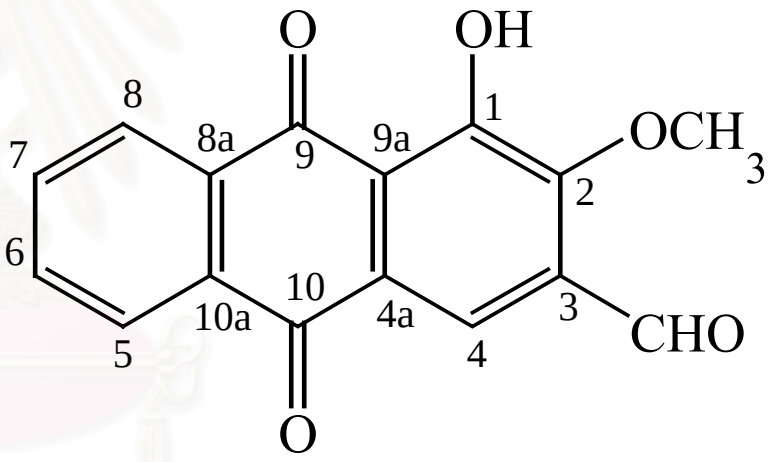
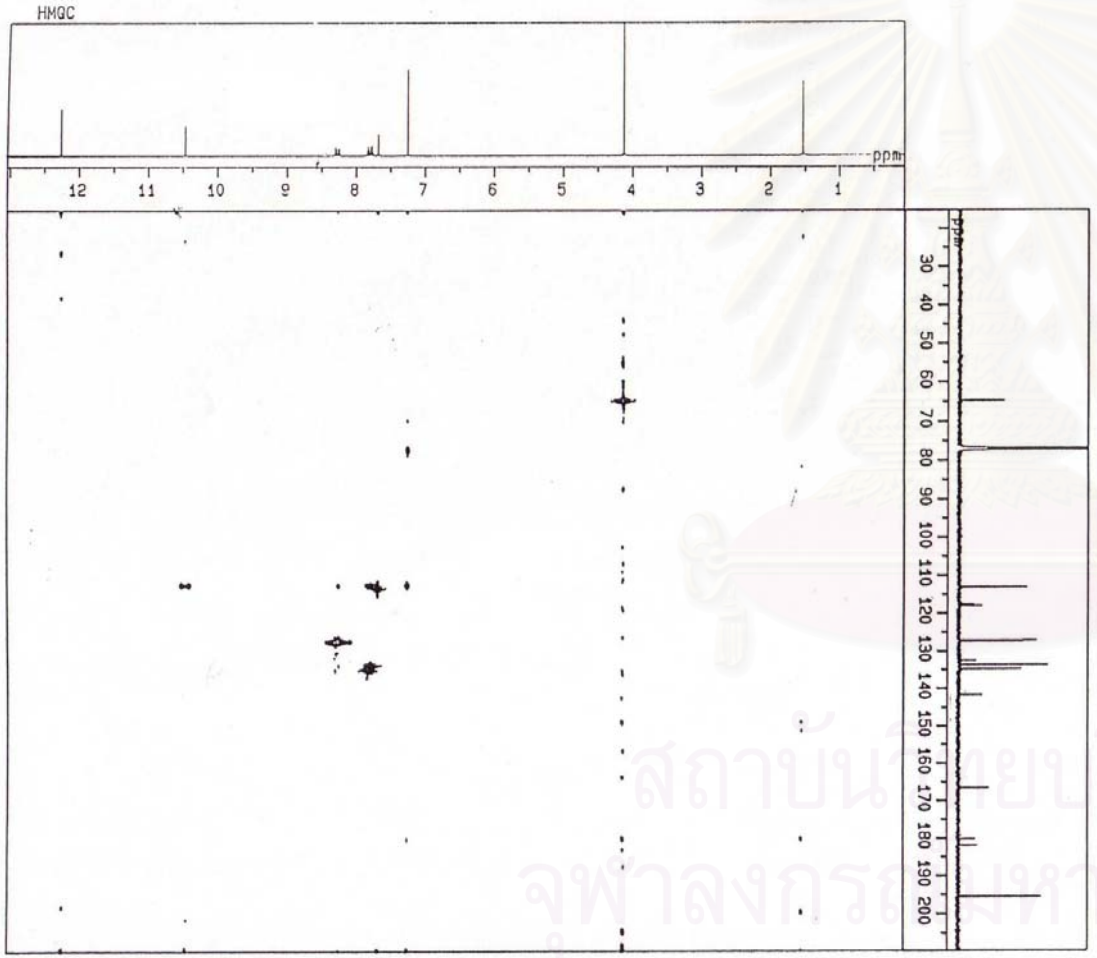


Figure 9b. The NOESY spectrum of Compound M in $CDCl_3$ (expanded)



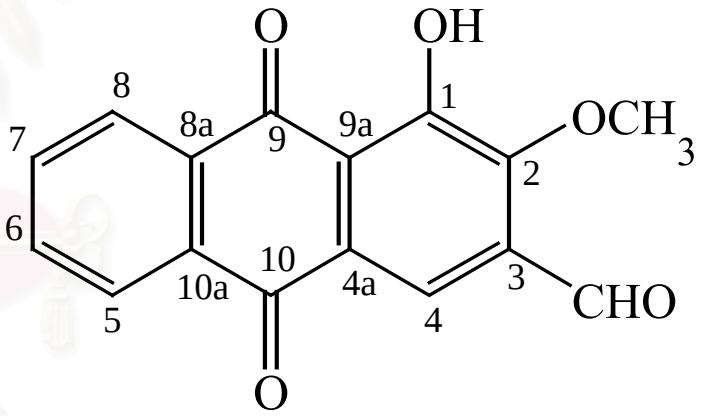
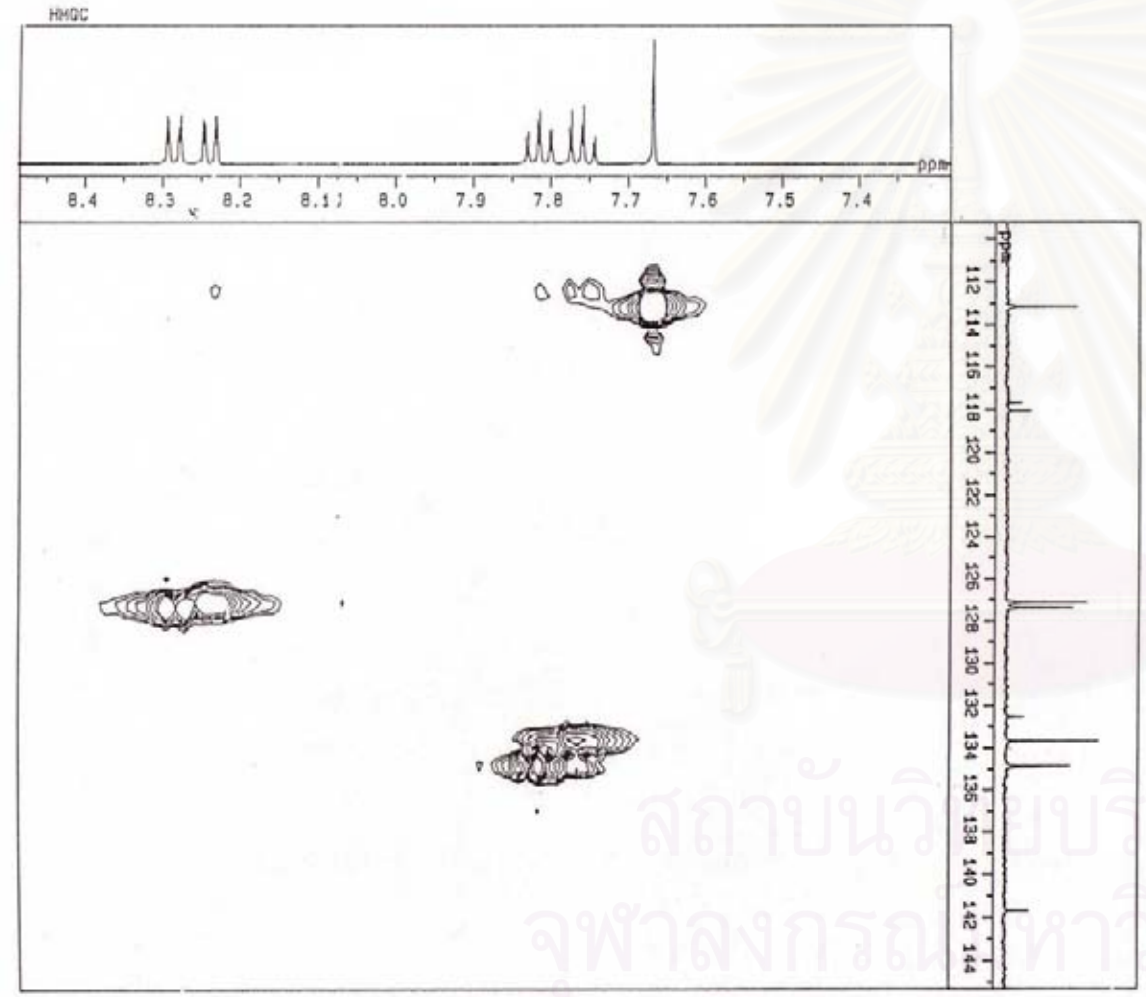


Figure 10a. The HMQC spectrum of Compound M in $CDCl_3$



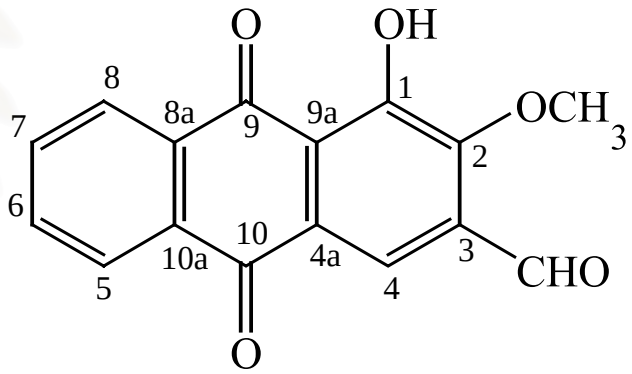
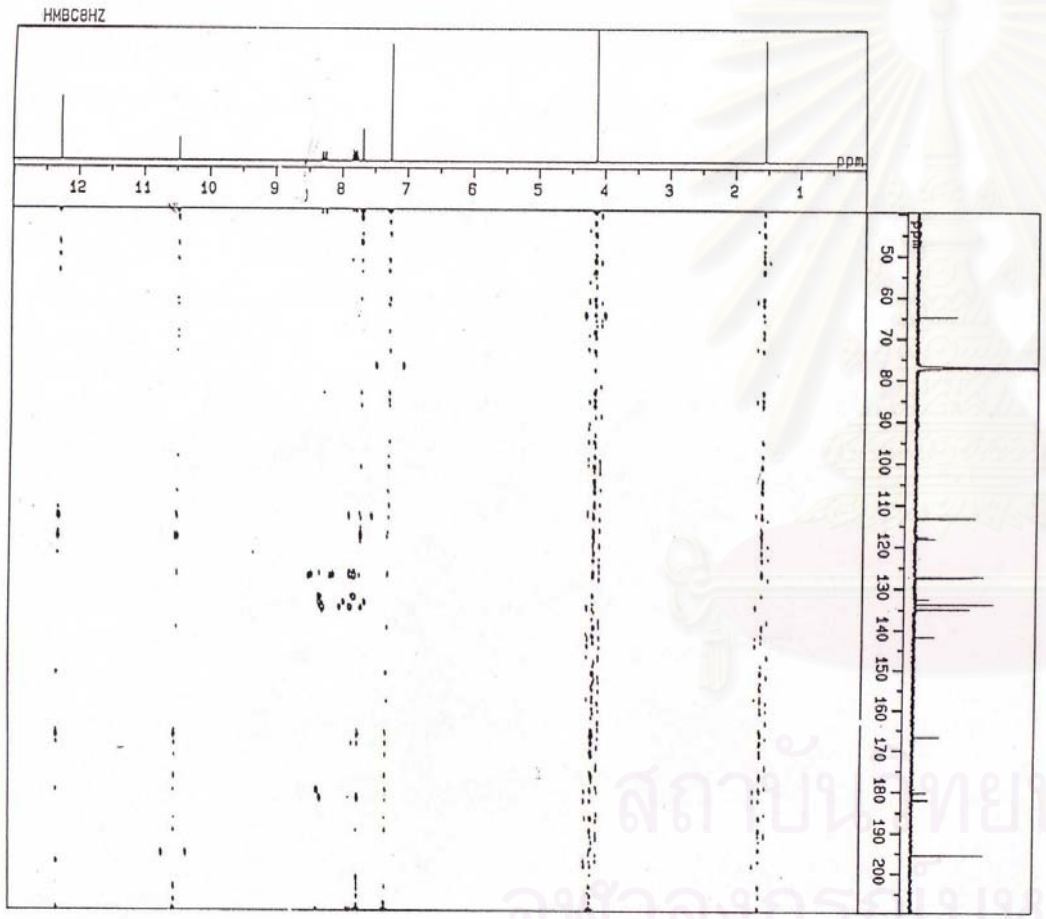


Figure 10b. The HMQC spectrum of Compound M in $CDCl_3$ (expanded)



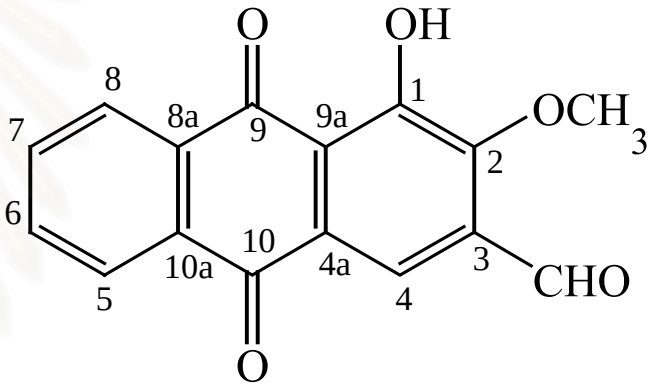
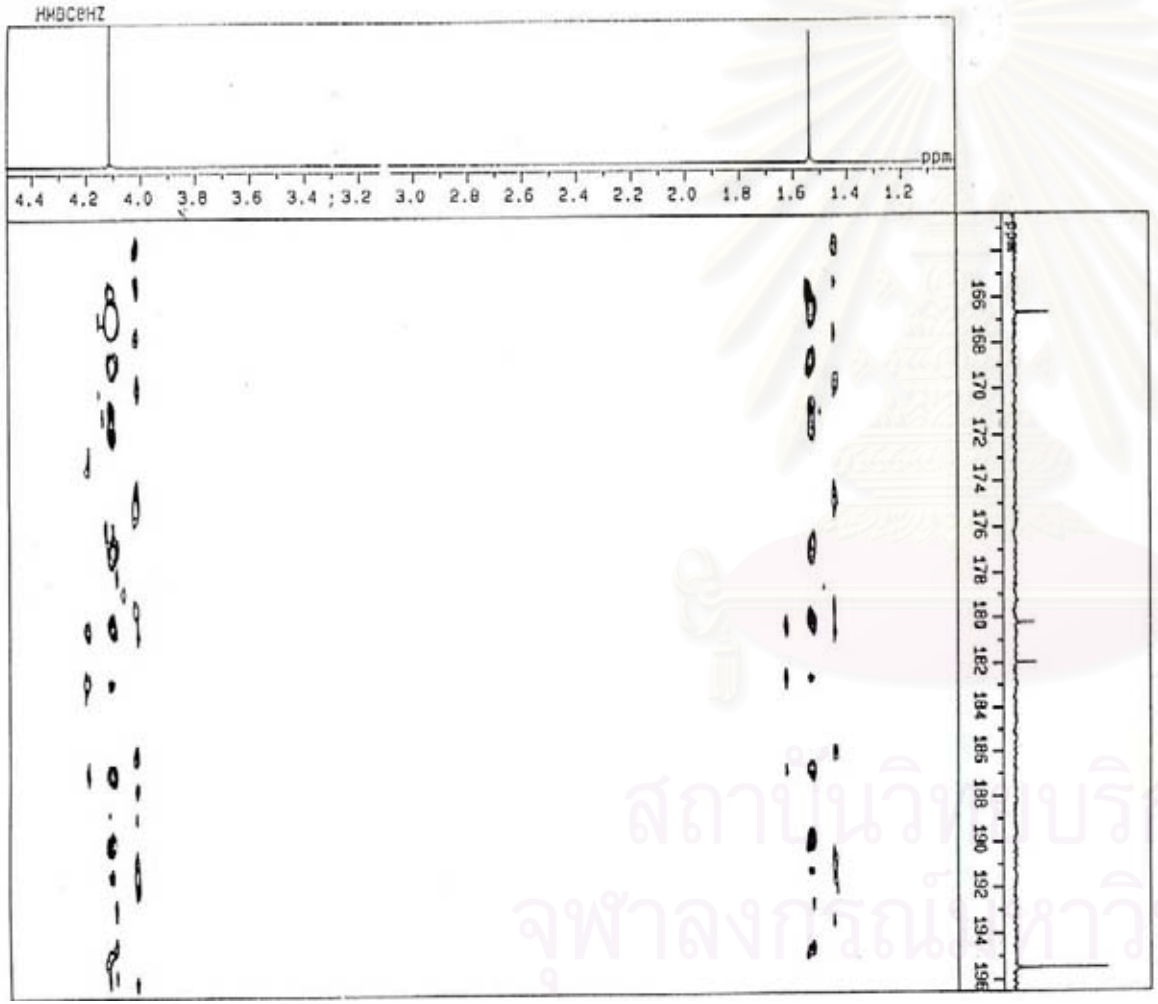


Figure 11a. The HMBC spectrum of Compound M in $CDCl_3$ (expanded)



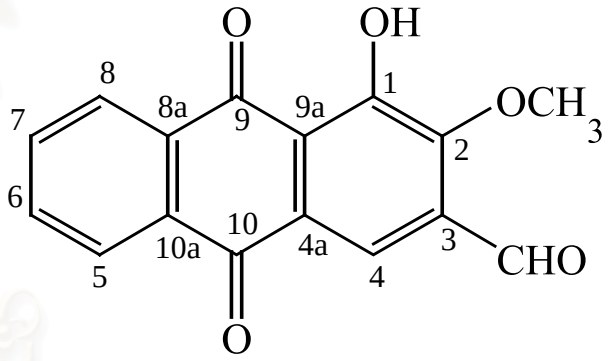
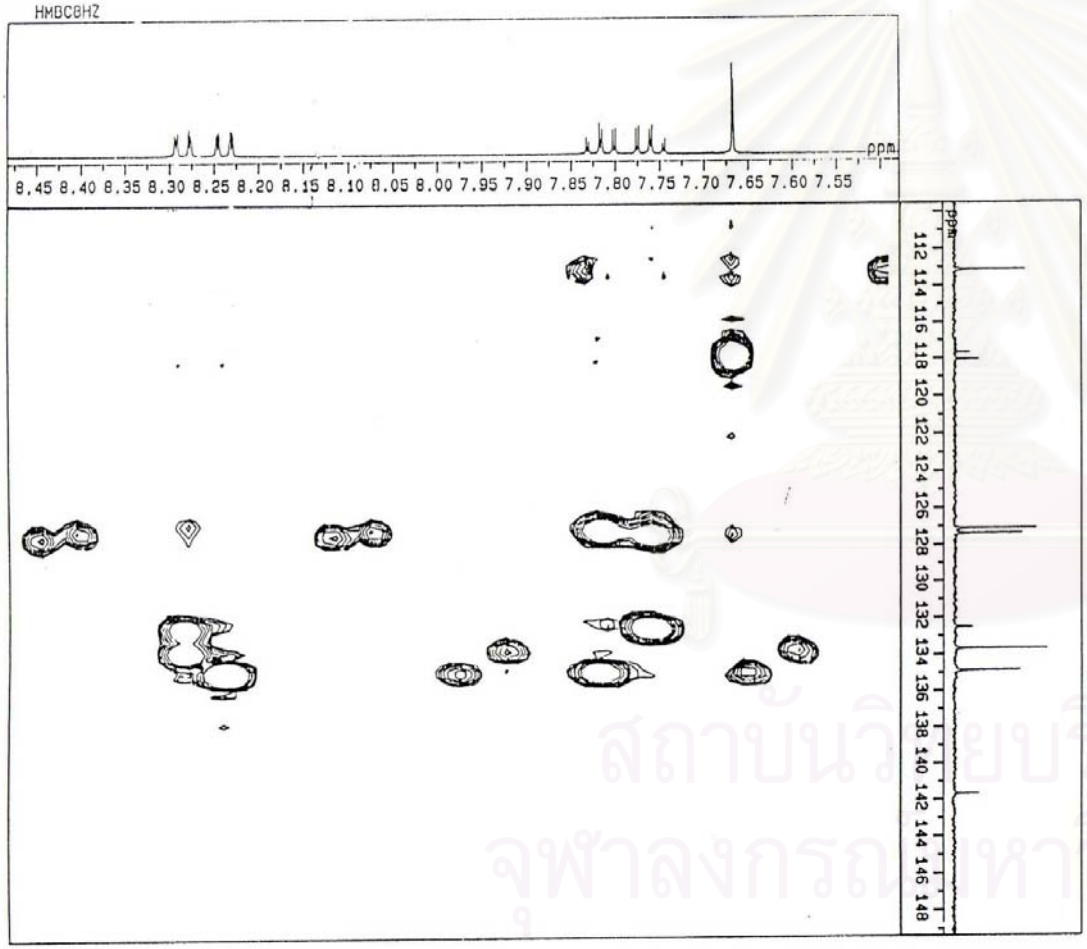


Figure 11b. The HMBC spectrum of Compound M in $CDCl_3$ (expanded)



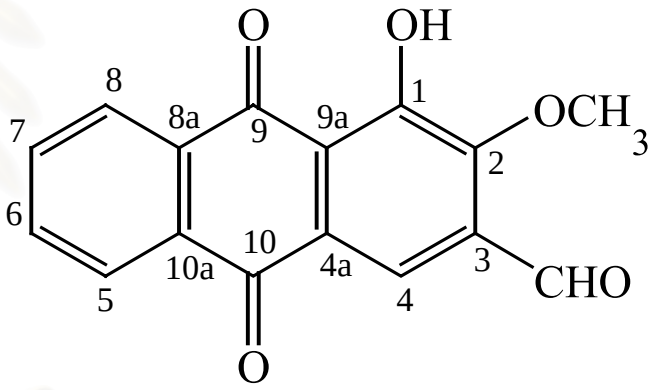


Figure 11c. The HMBC spectrum of Compound M in $CDCl_3$ (expanded)

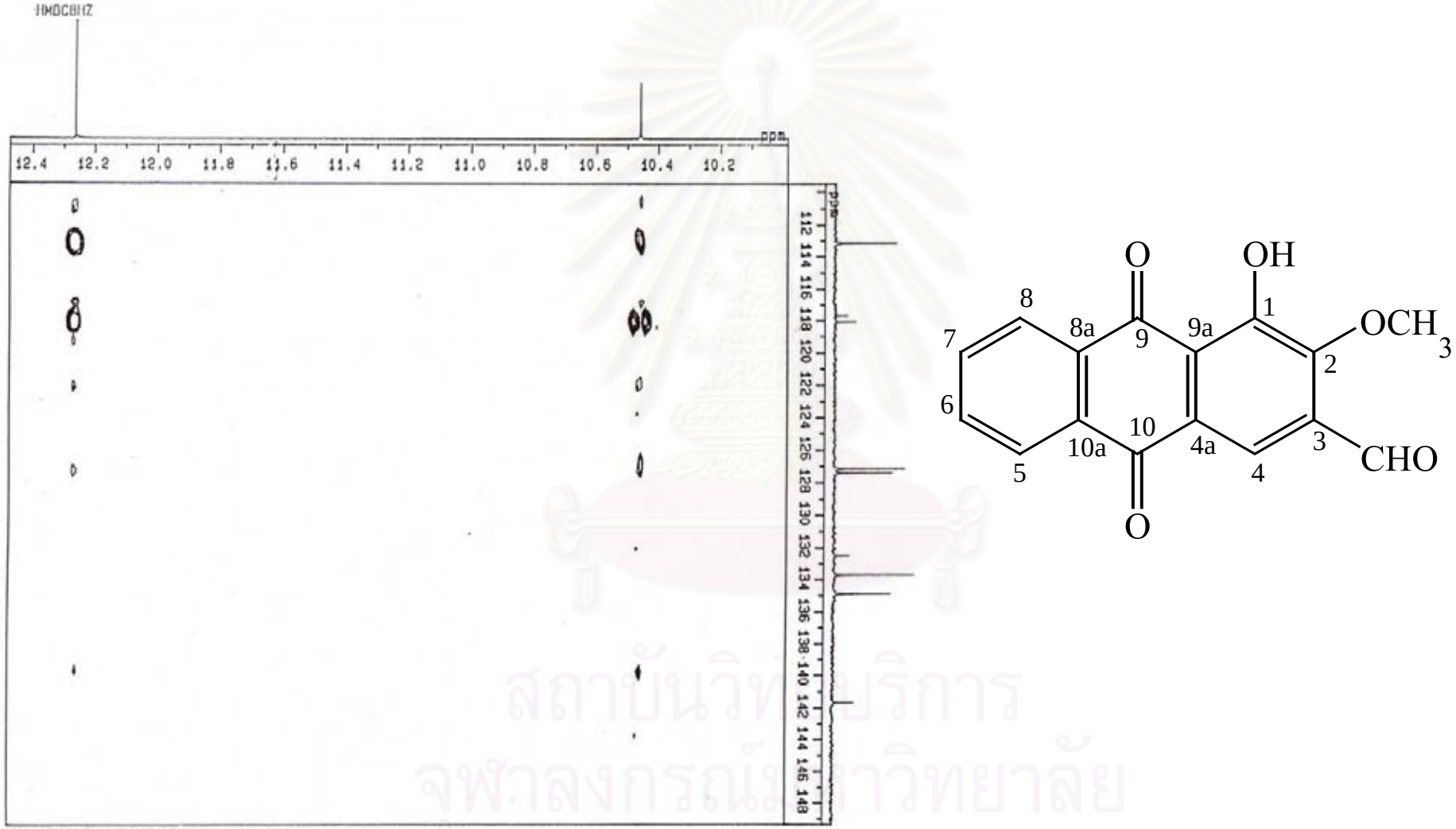
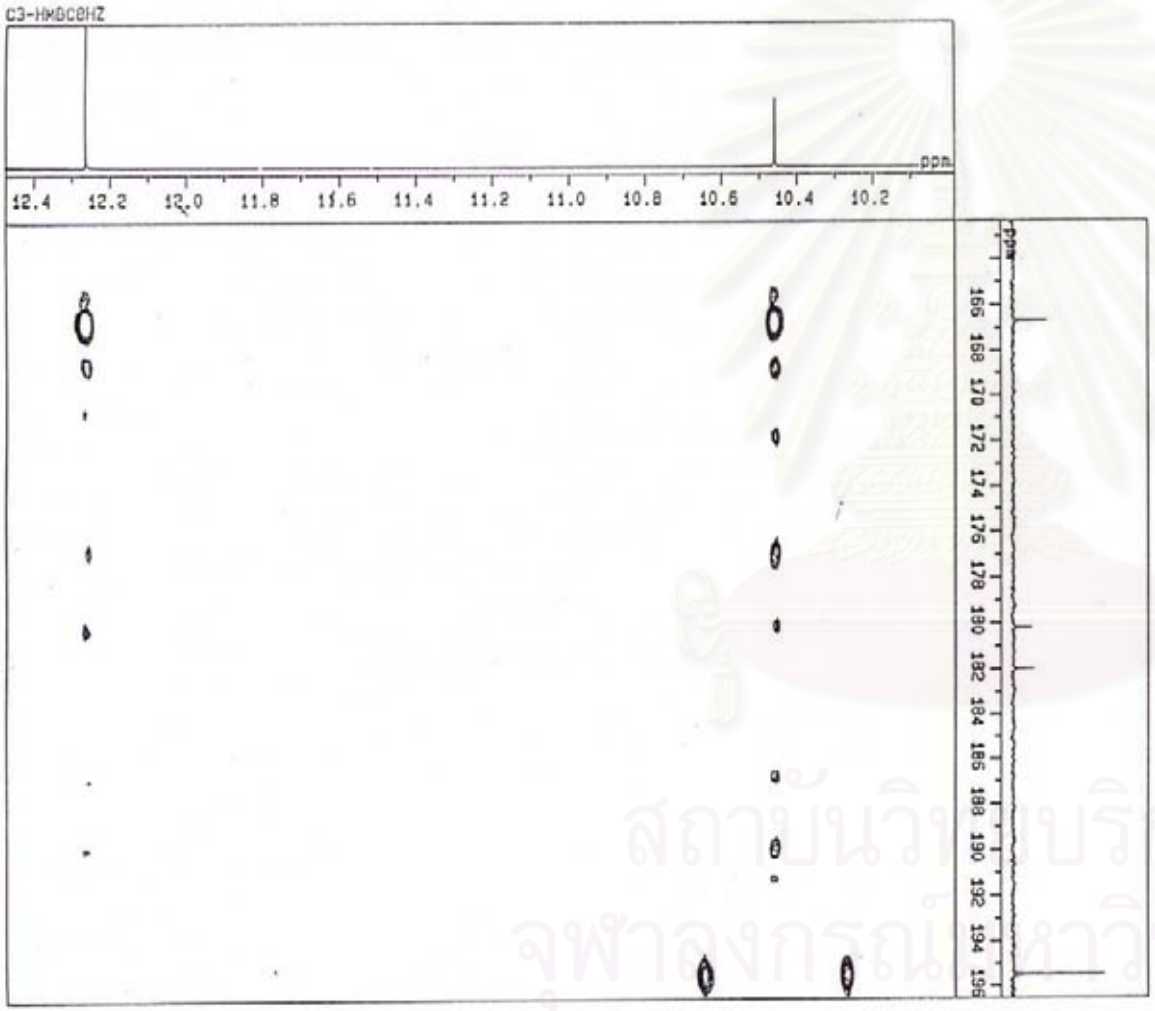


Figure 11d. The HMBC spectrum of Compound M in $CDCl_3$ (expanded)



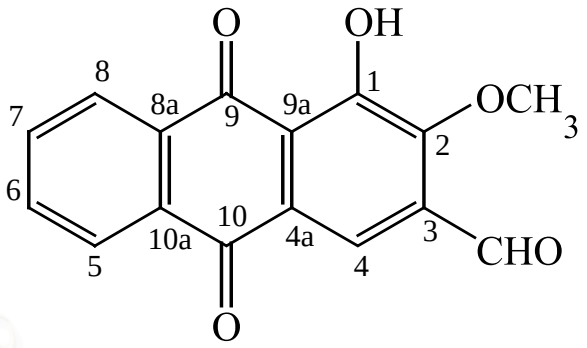


Figure 11e. The HMBC spectrum of Compound M in $CDCl_3$ (expanded)

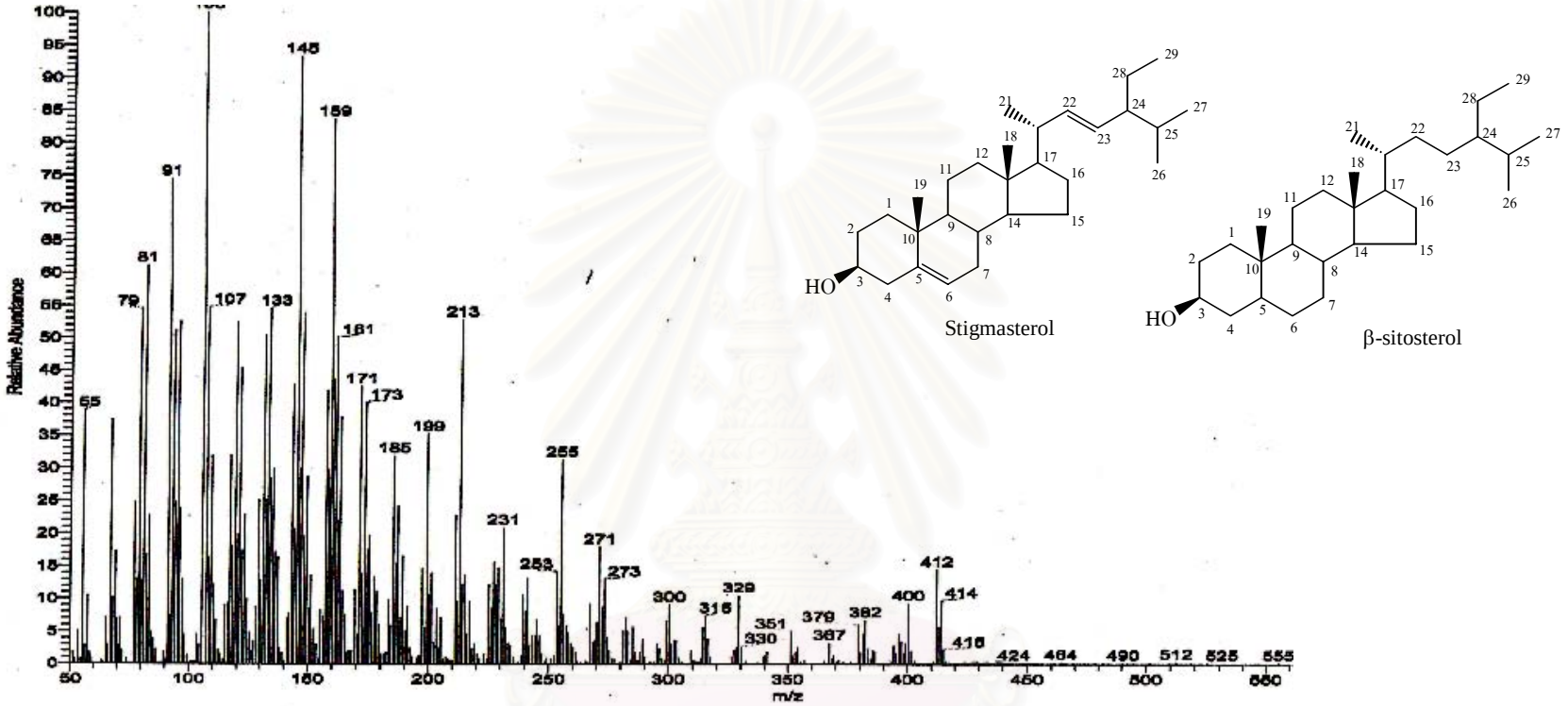


Figure 12. EI Mass spectrum of Compound H-2

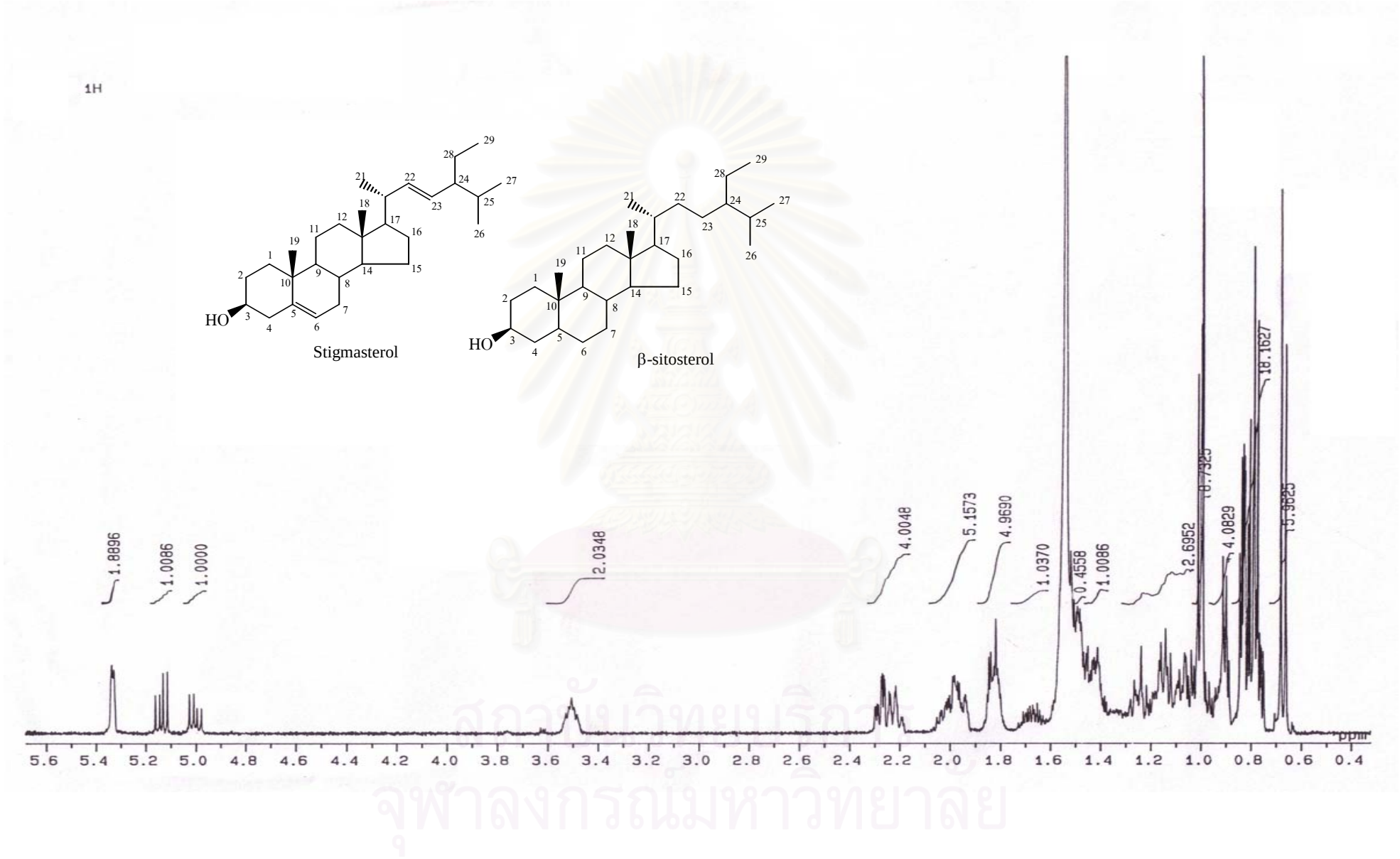


Figure 13. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound H-2 in $CDCl_3$um of Compound H-2

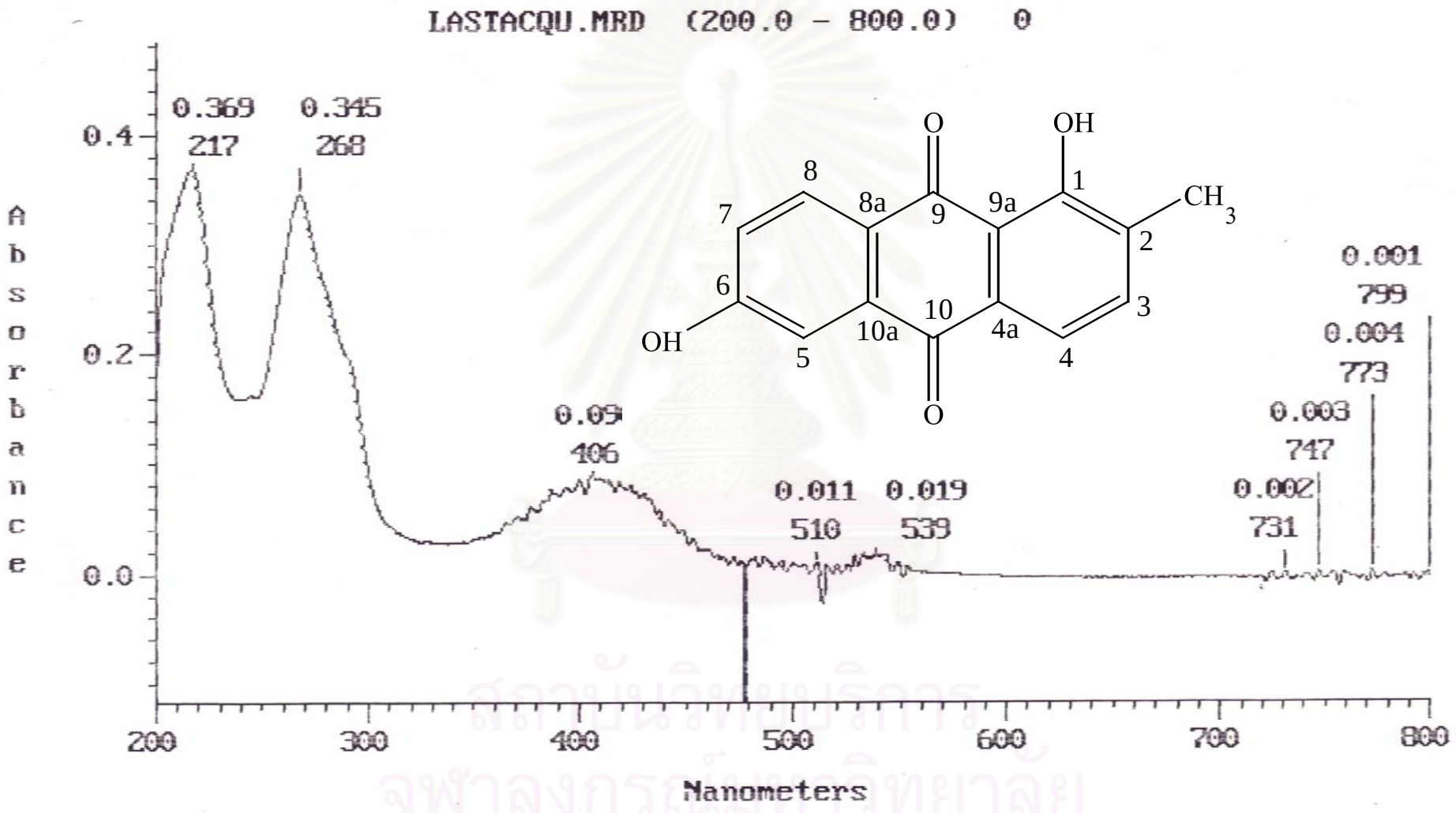


Figure 14. Ultraviolet absorption spectrum of Compound H-3 (in MeOH)

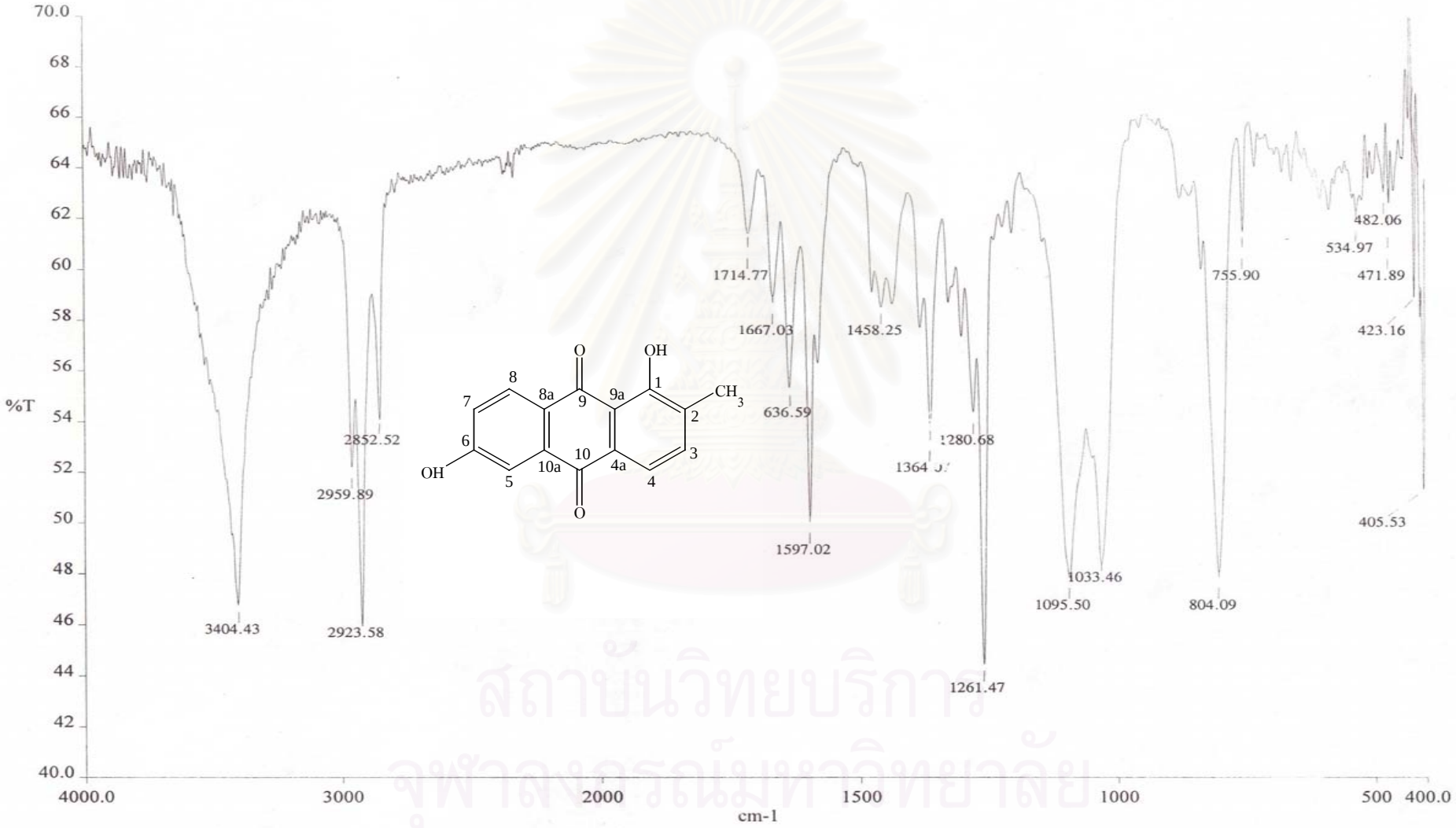


Figure 15. Infrared absorption spectrum of Compound H-3 (KBr)

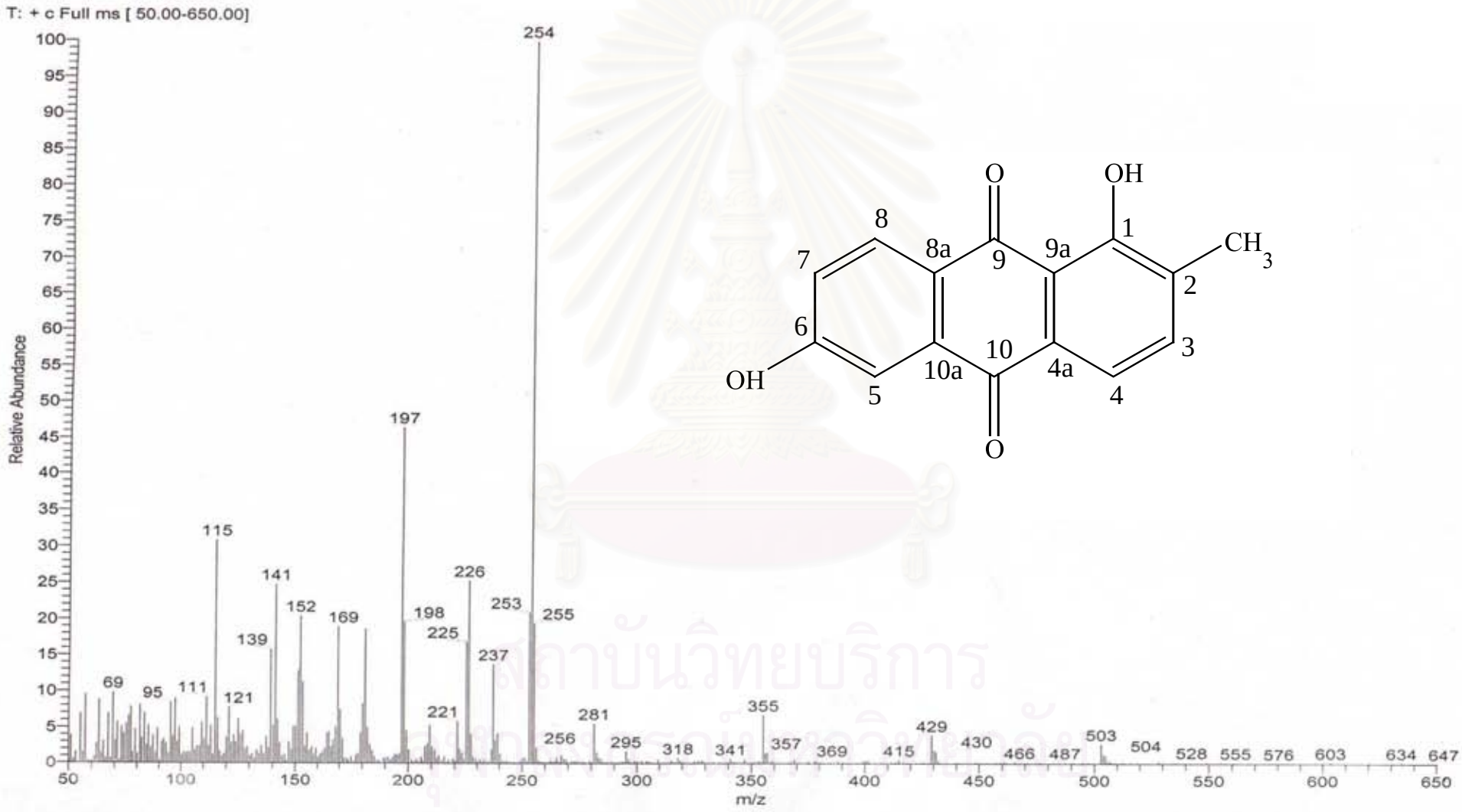


Figure 16. EI Mass spectrum of Compound H-3

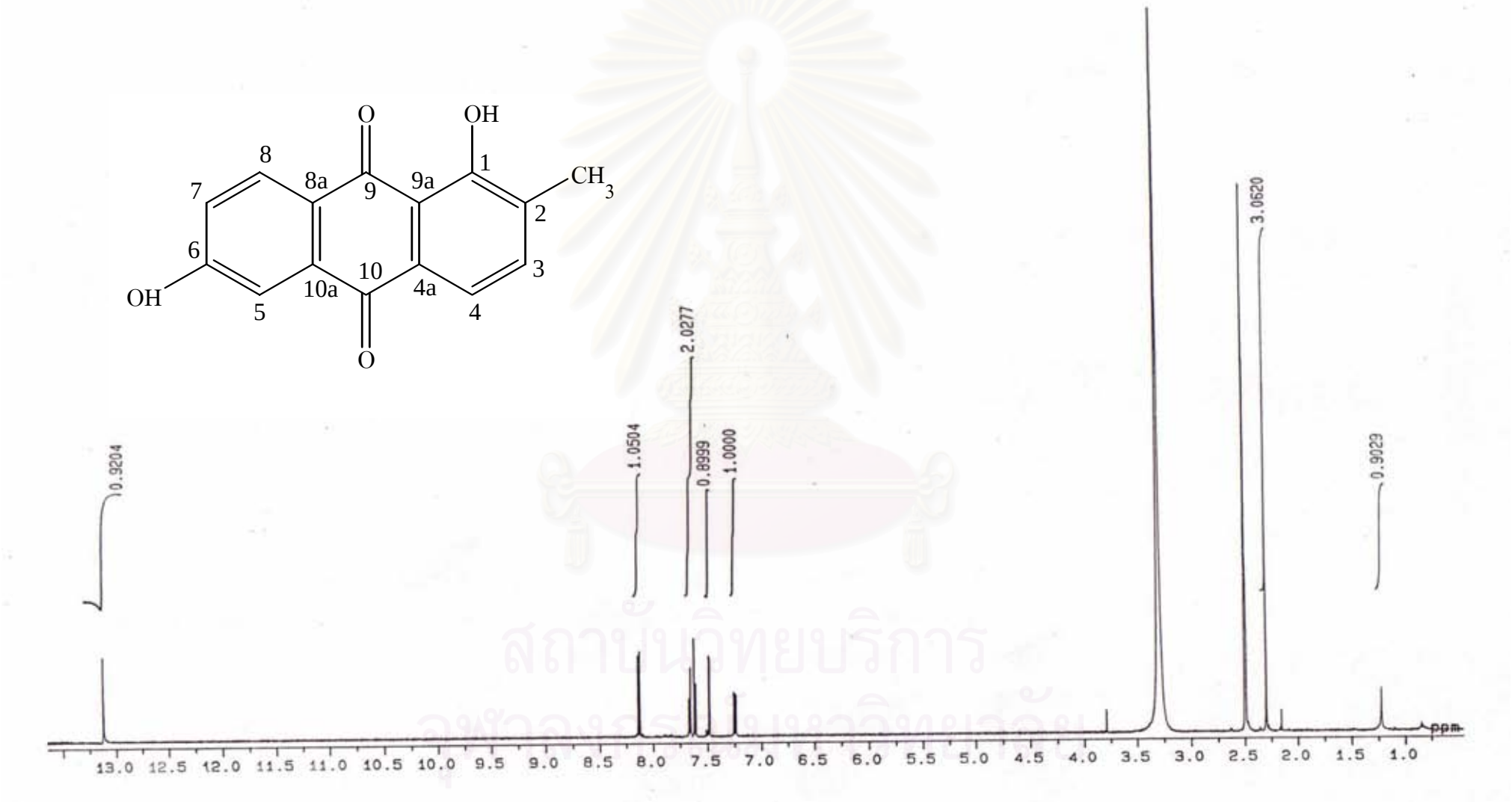


Figure 17a. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound H-3 in DMSO-d_6

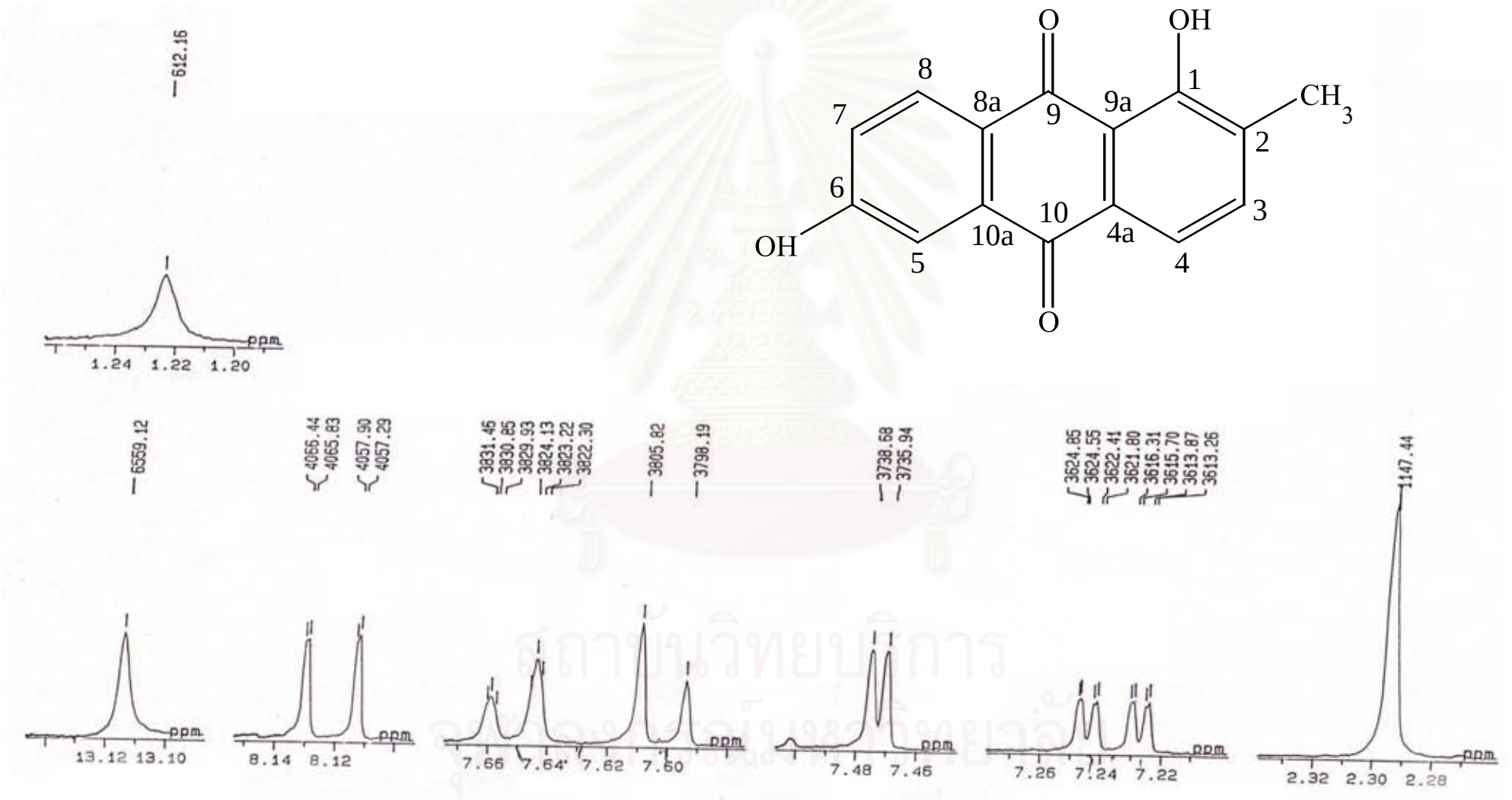


Figure 17b. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound H-3 in DMSO-d_6 (expanded)

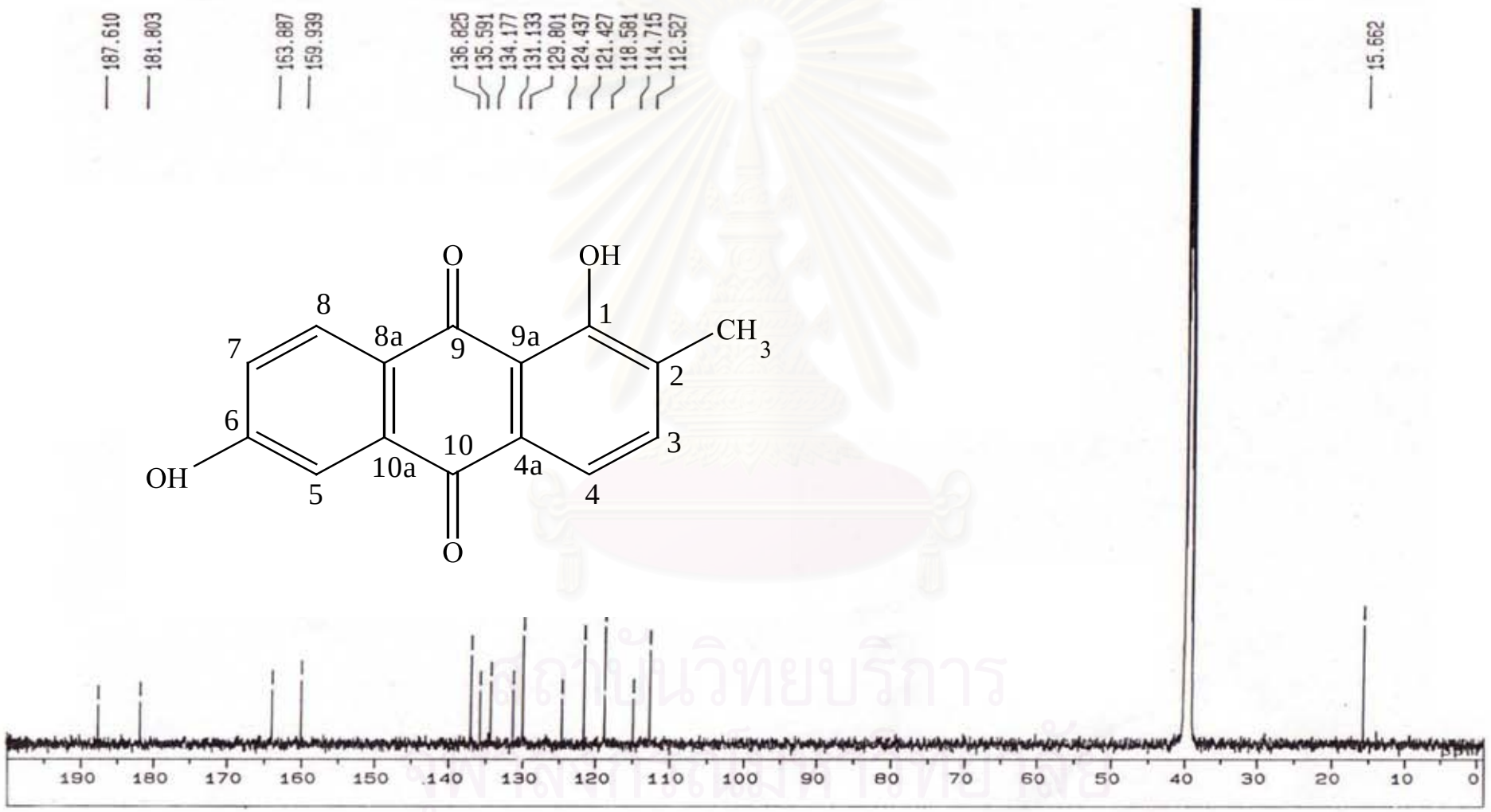


Figure 18. The ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound H-3 in DMSO-d_6

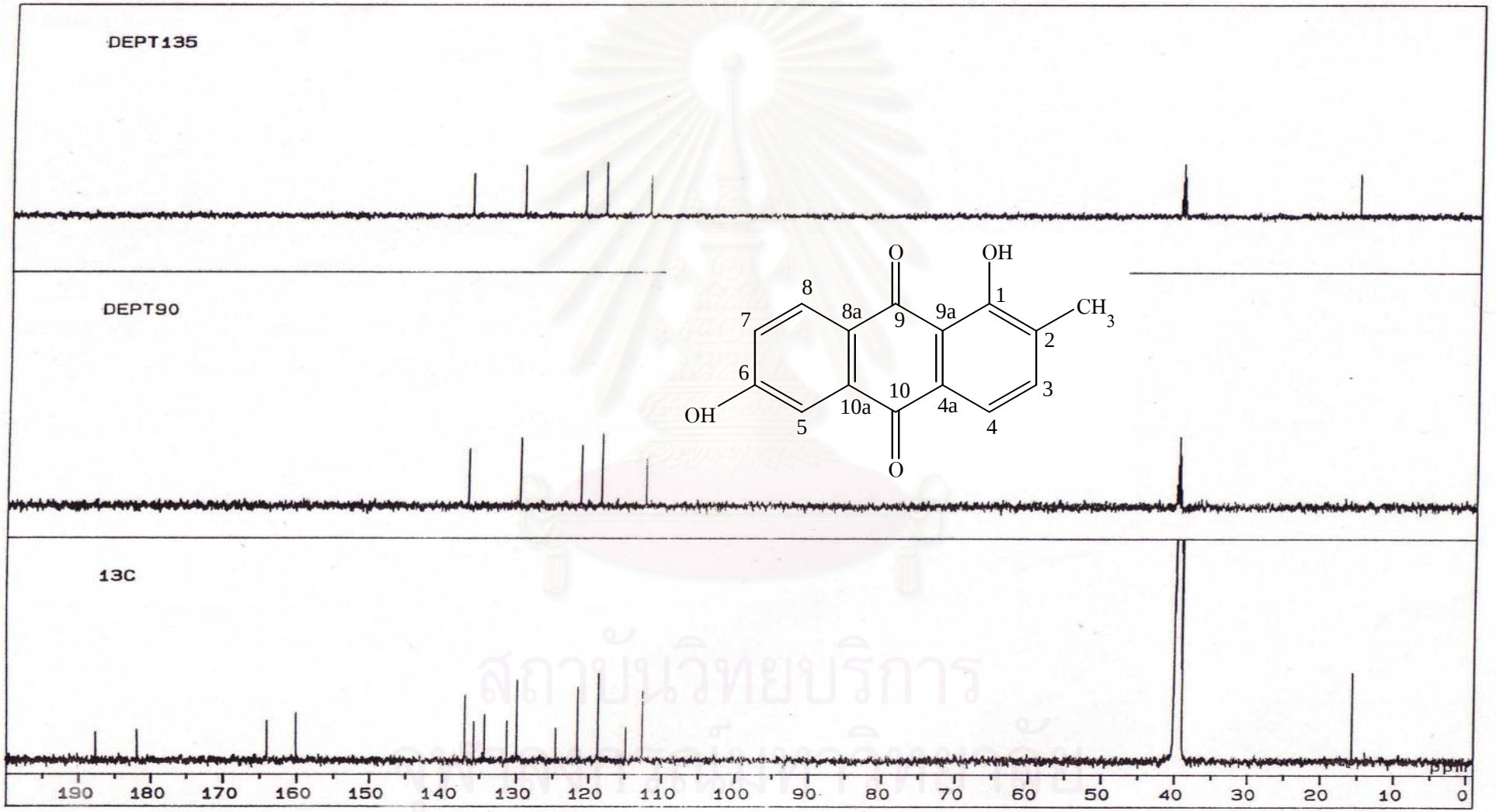


Figure 19. The DEPT-135, 90 and ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound H-3 in DMSO-d_6

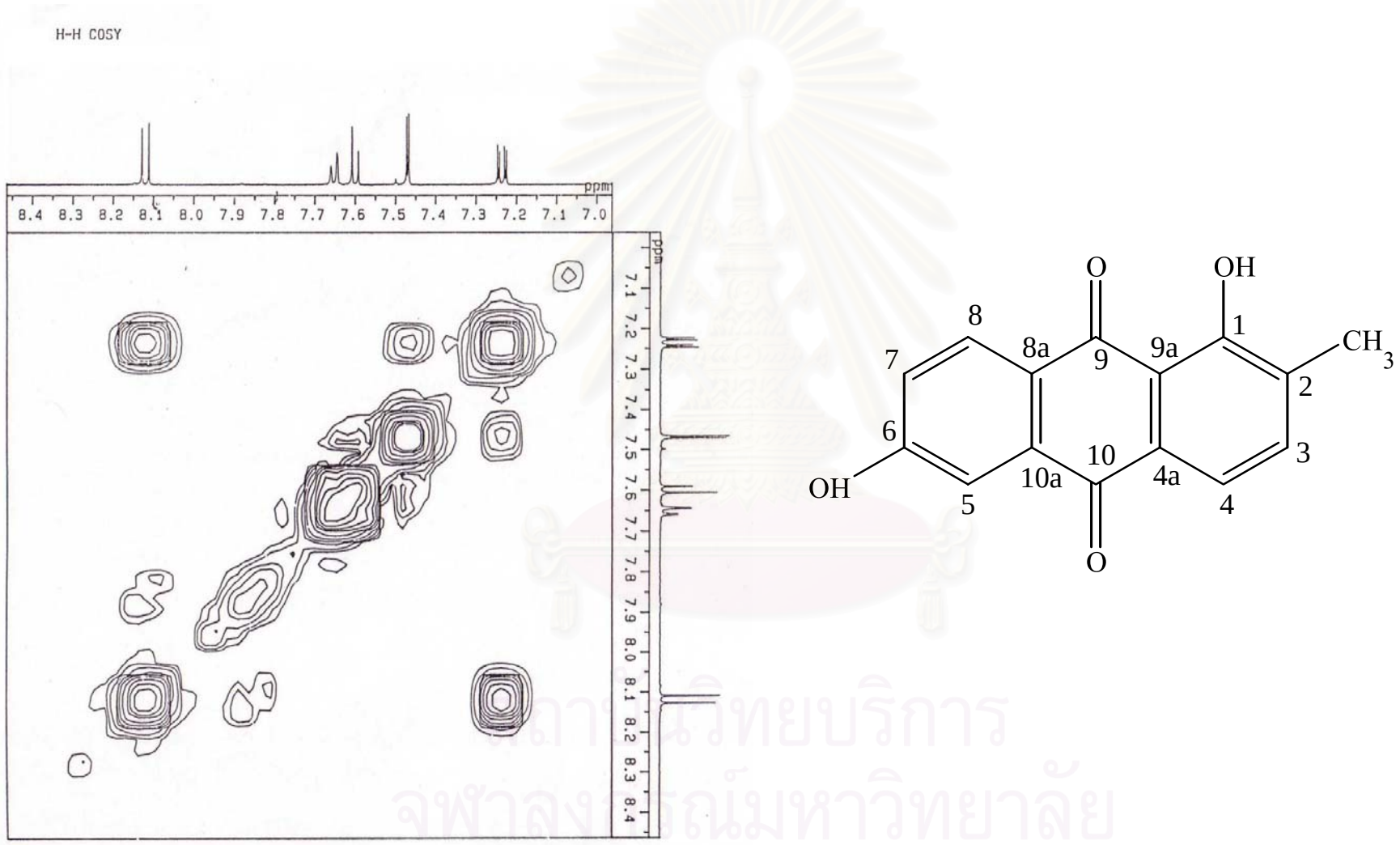


Figure 20. The ^{1}H-^{1}H COSY spectrum of Compound H-3 in DMSO-d_6

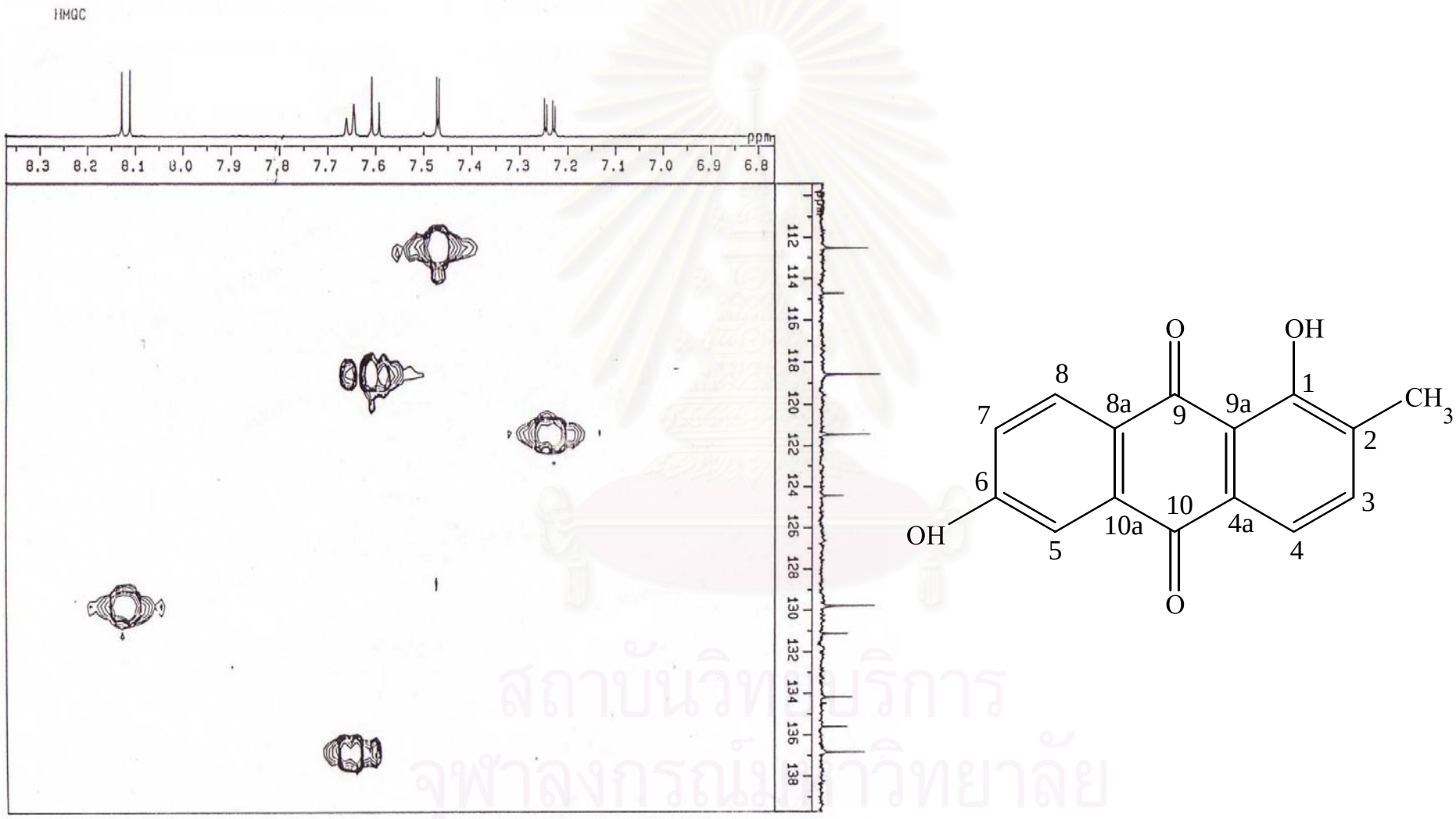
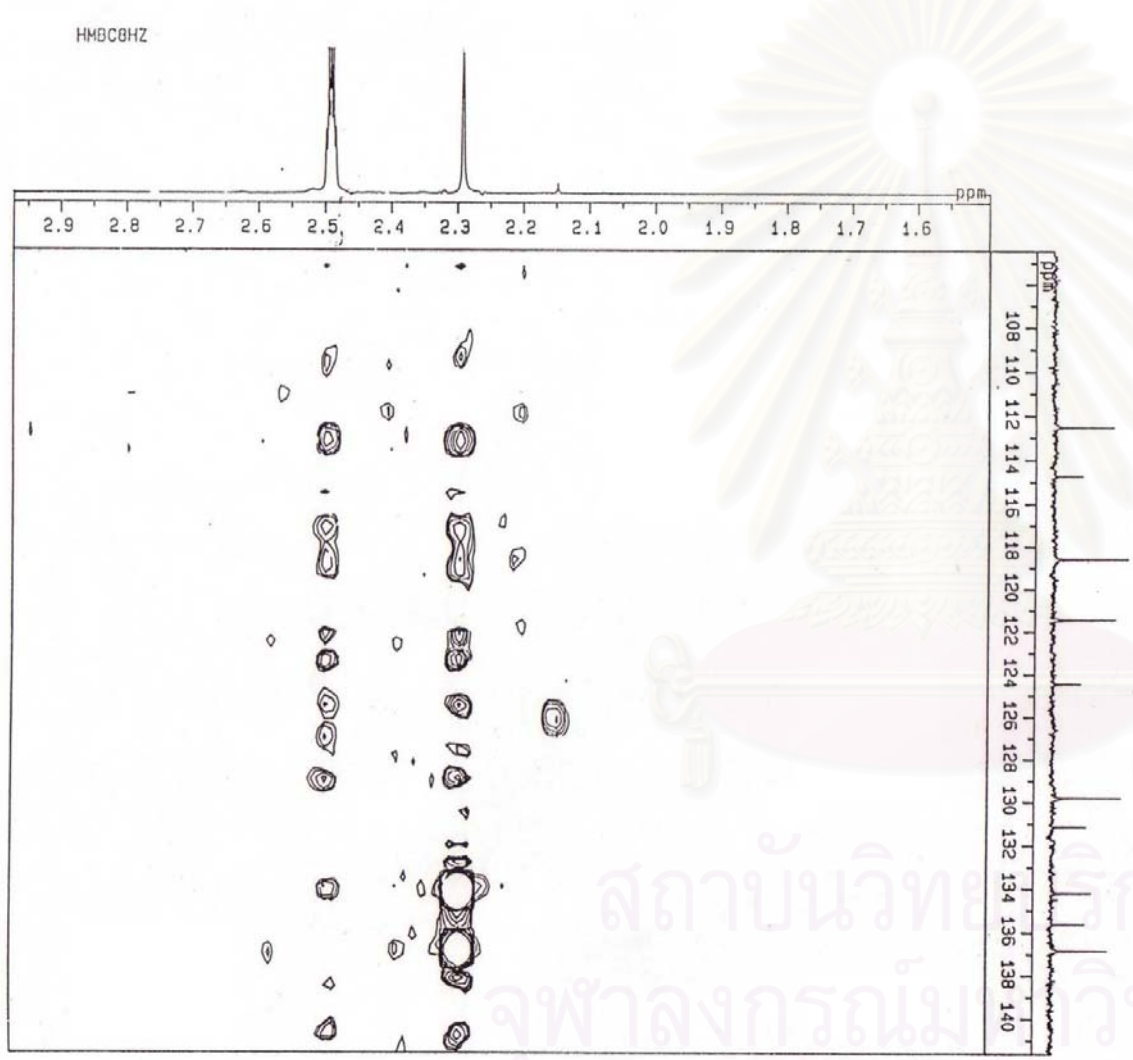


Figure 21 The HMQC spectrum of Compound H-3 in DMSO-d_6



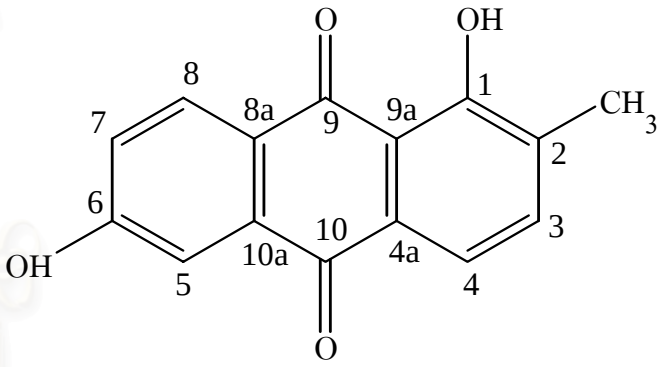


Figure 22a. The HMBC spectrum of Compound H-3 in DMSO-d_6

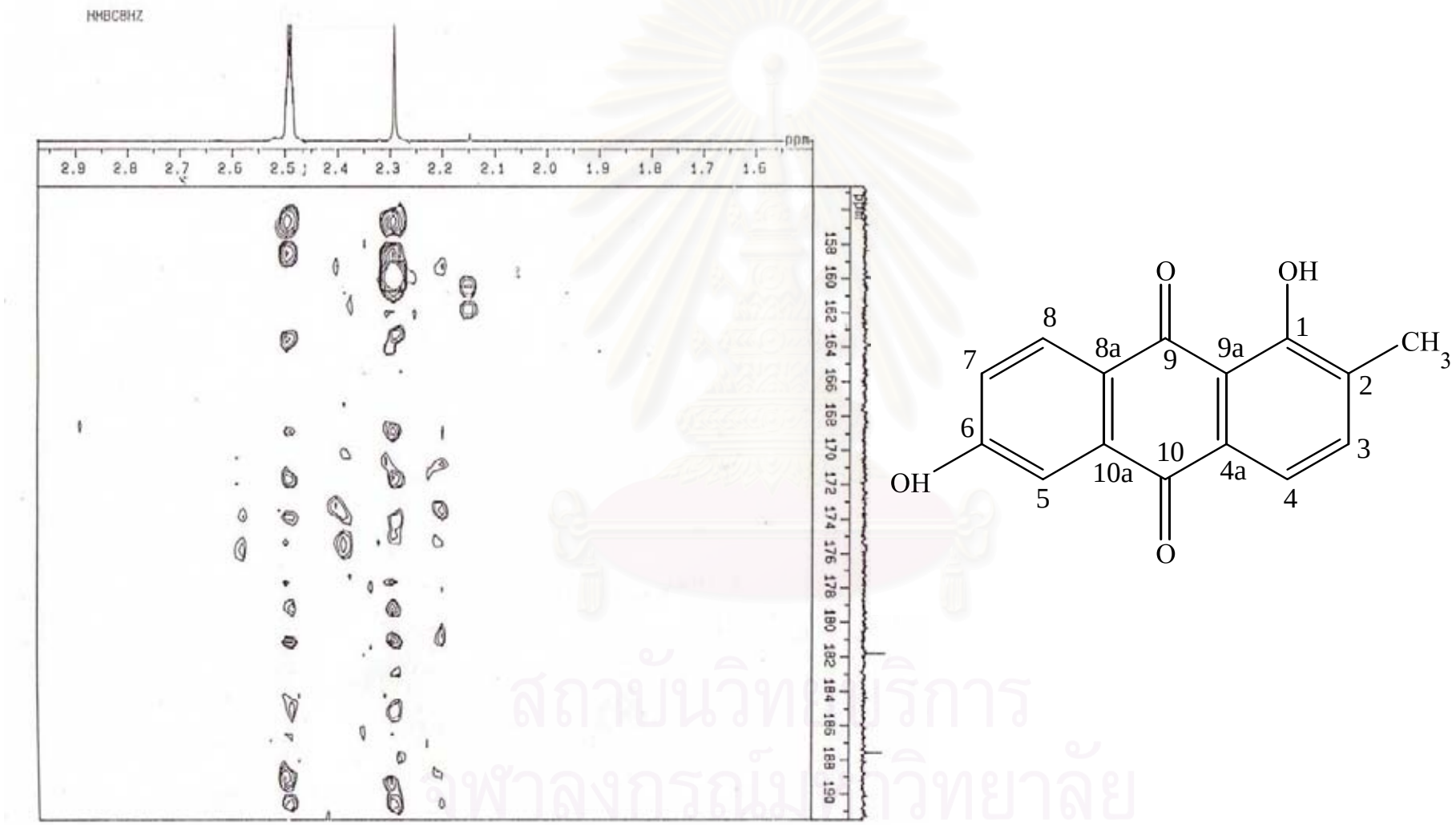
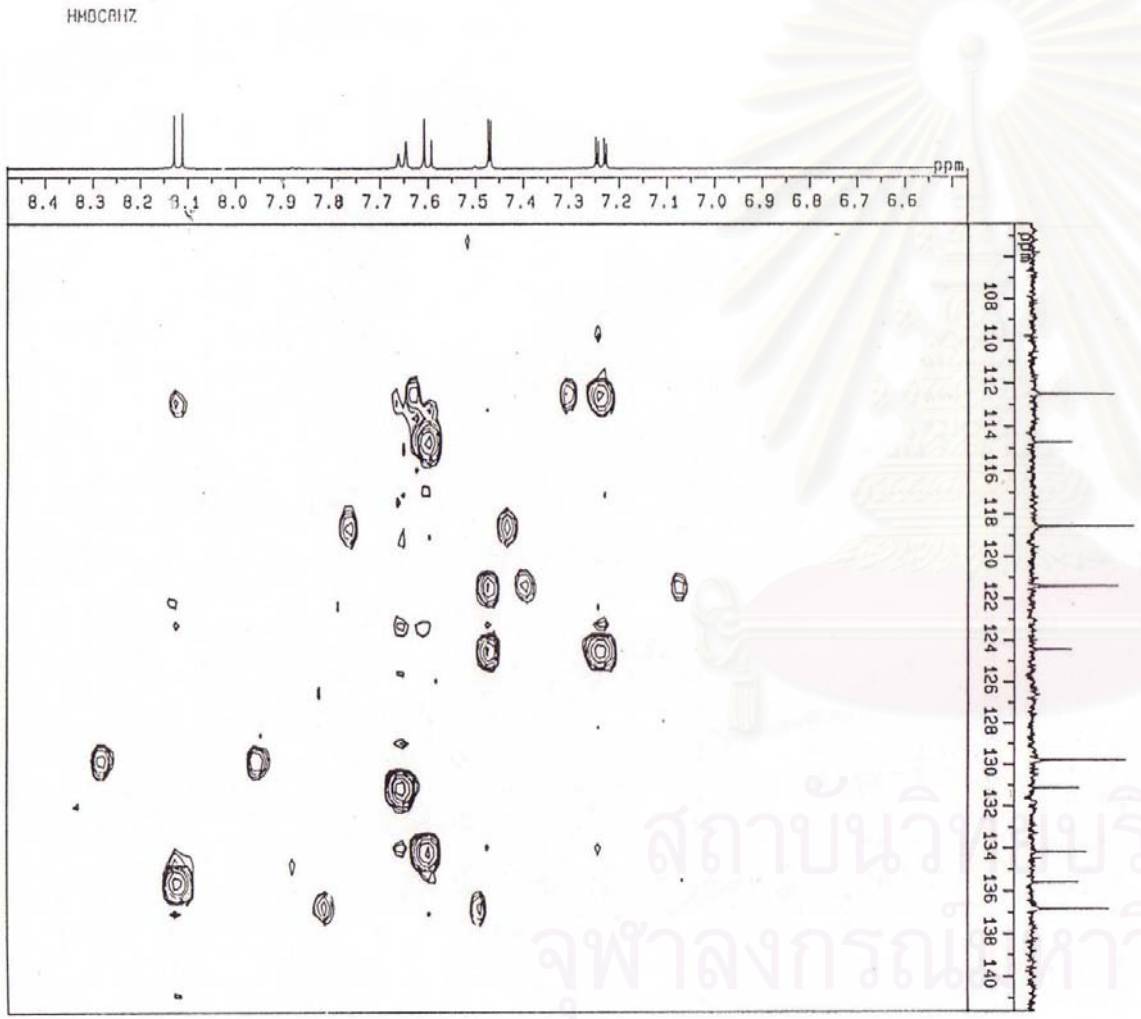


Figure 22b. The HMBC spectrum of Compound H-3 in DMSO-d_6



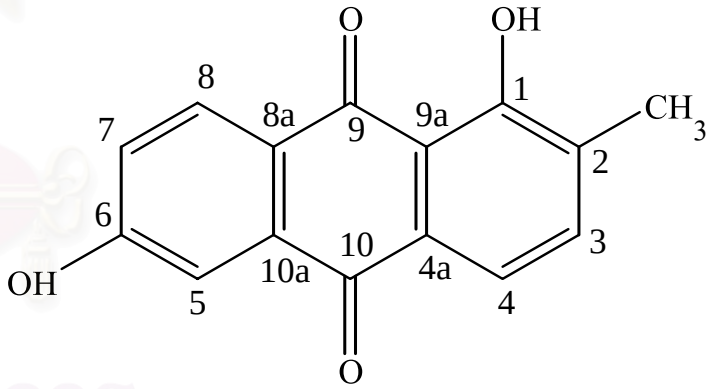
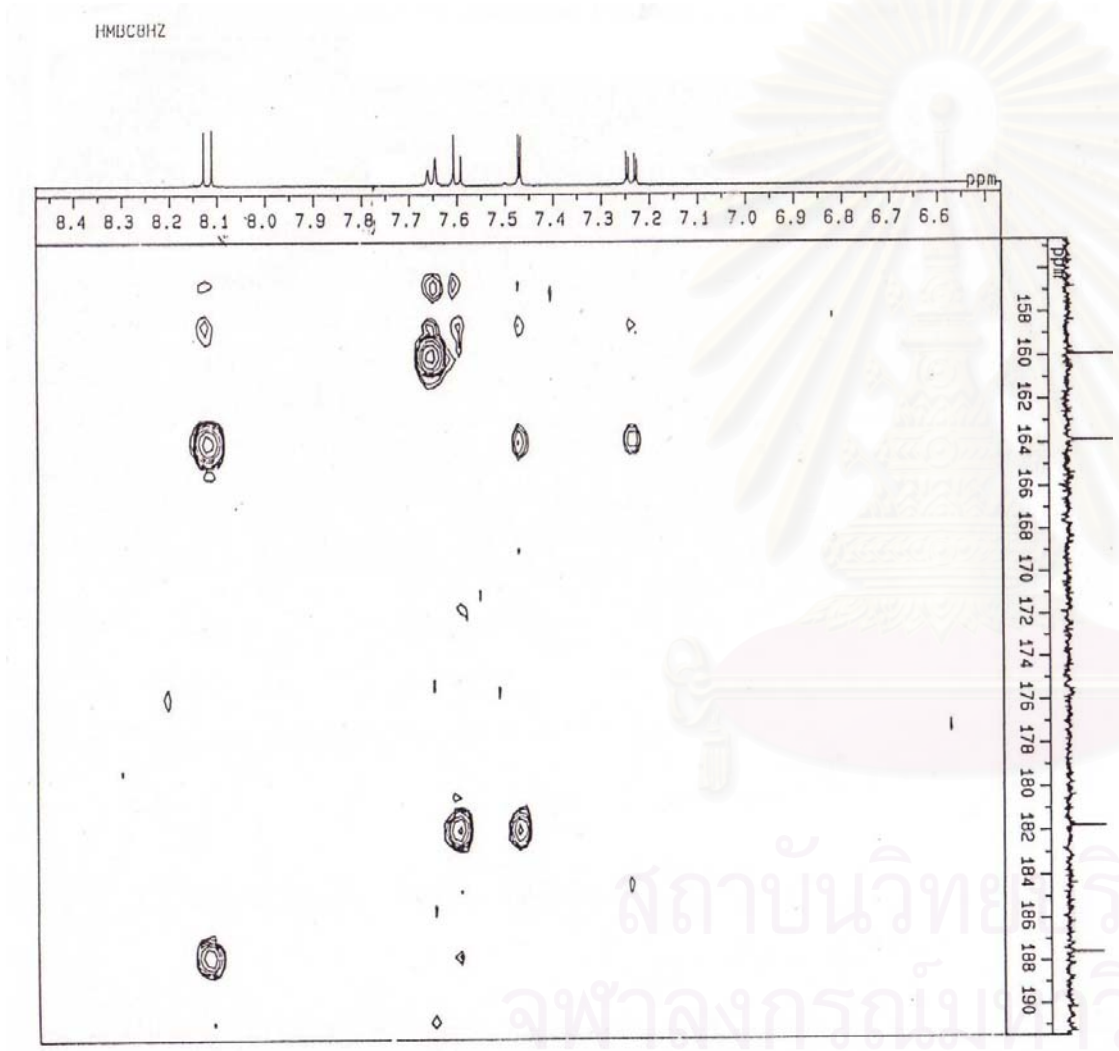


Figure 22c. The HMBC spectrum of Compound H-3 in DMSO-d_6



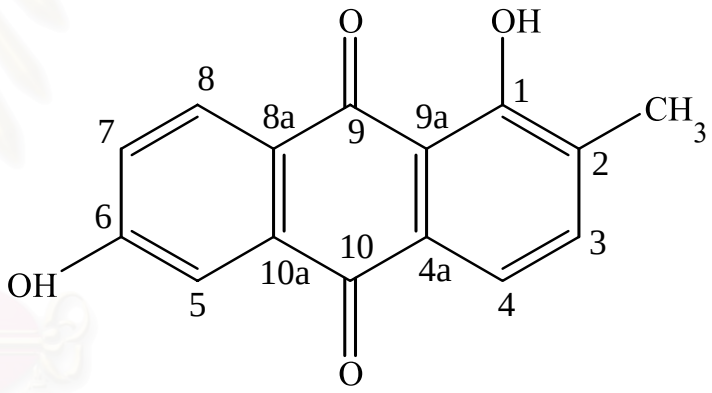
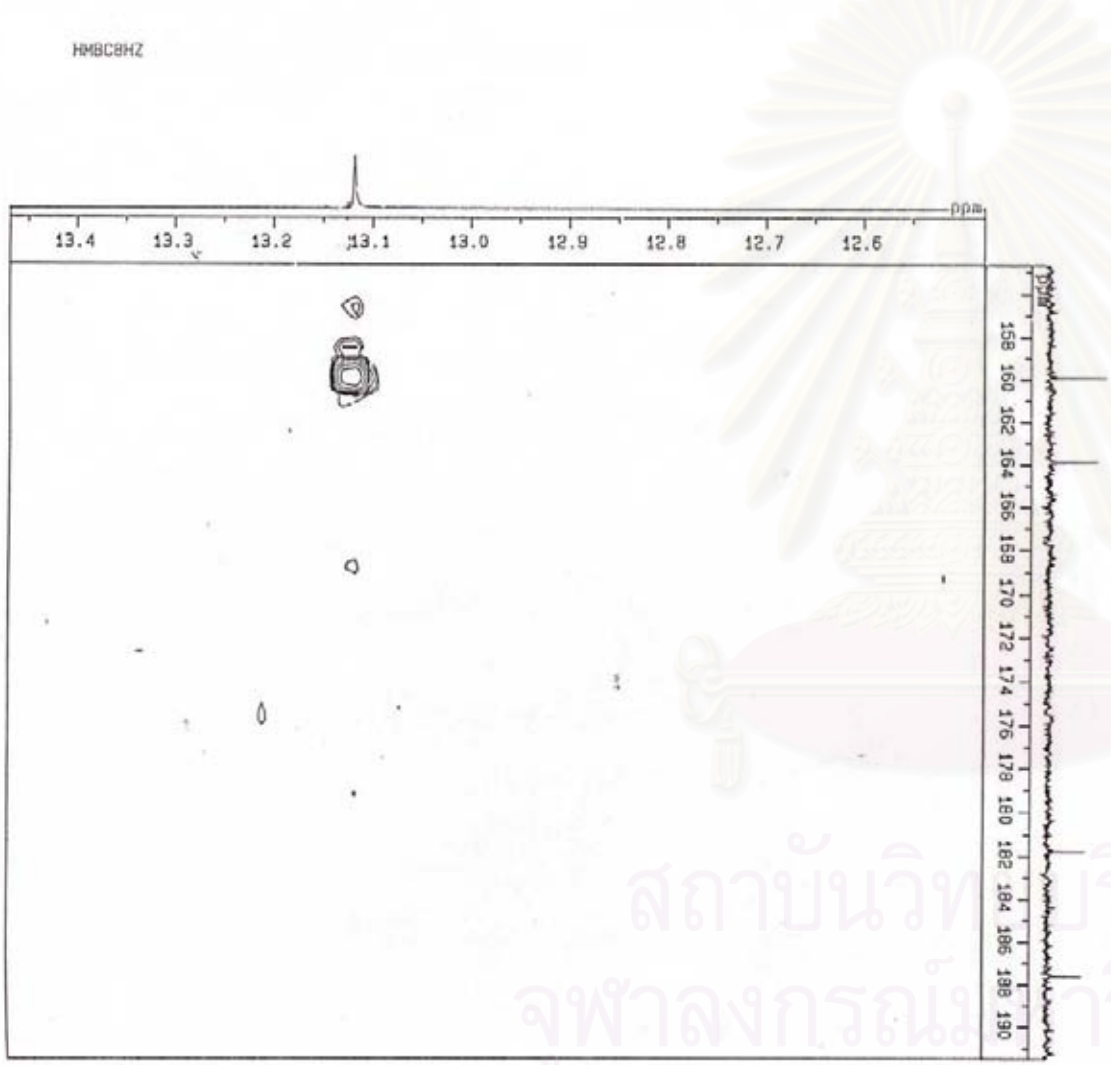


Figure 22d. The HMBC spectrum of Compound H-3 in DMSO-d_6



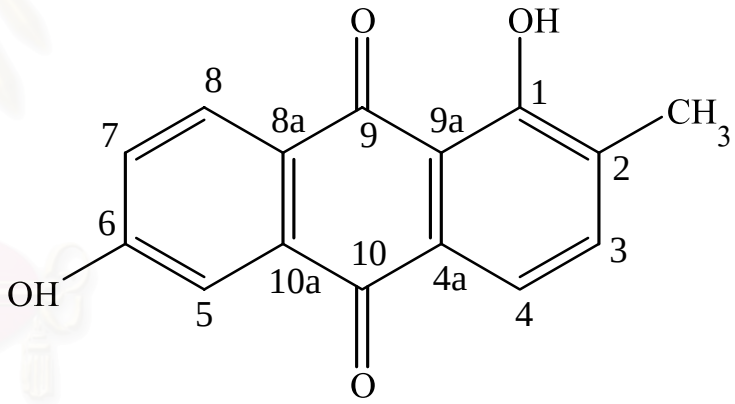
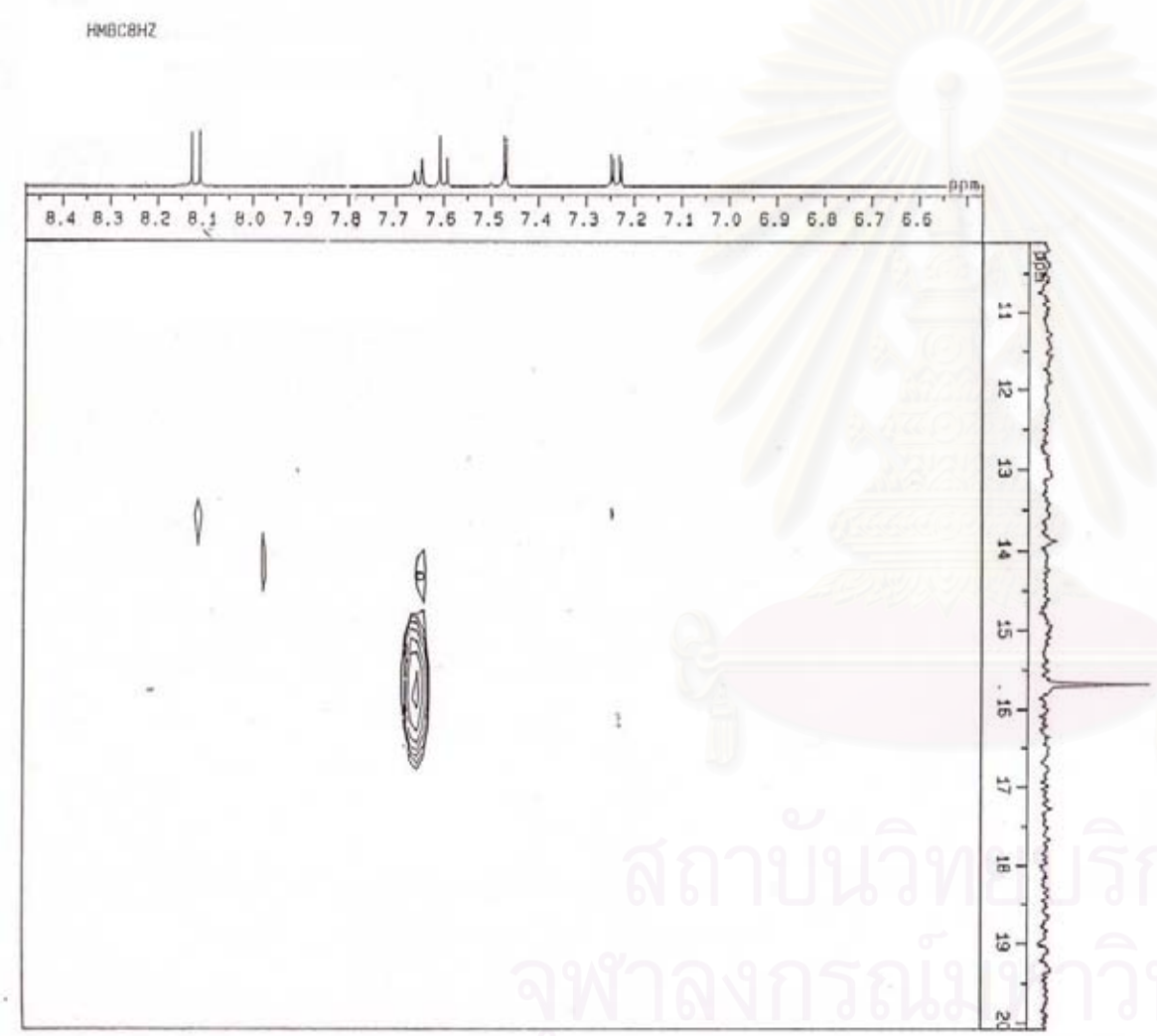


Figure 22e. The HMBC spectrum of Compound H-3 in DMSO-d_6



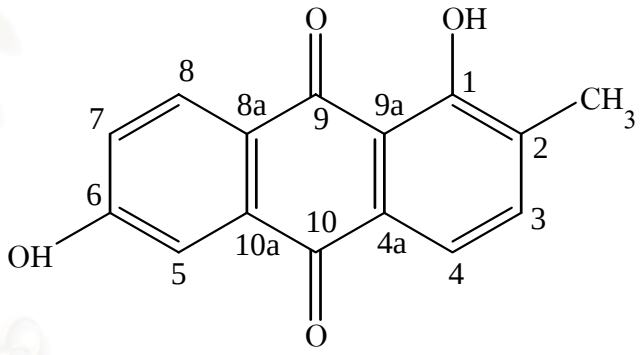
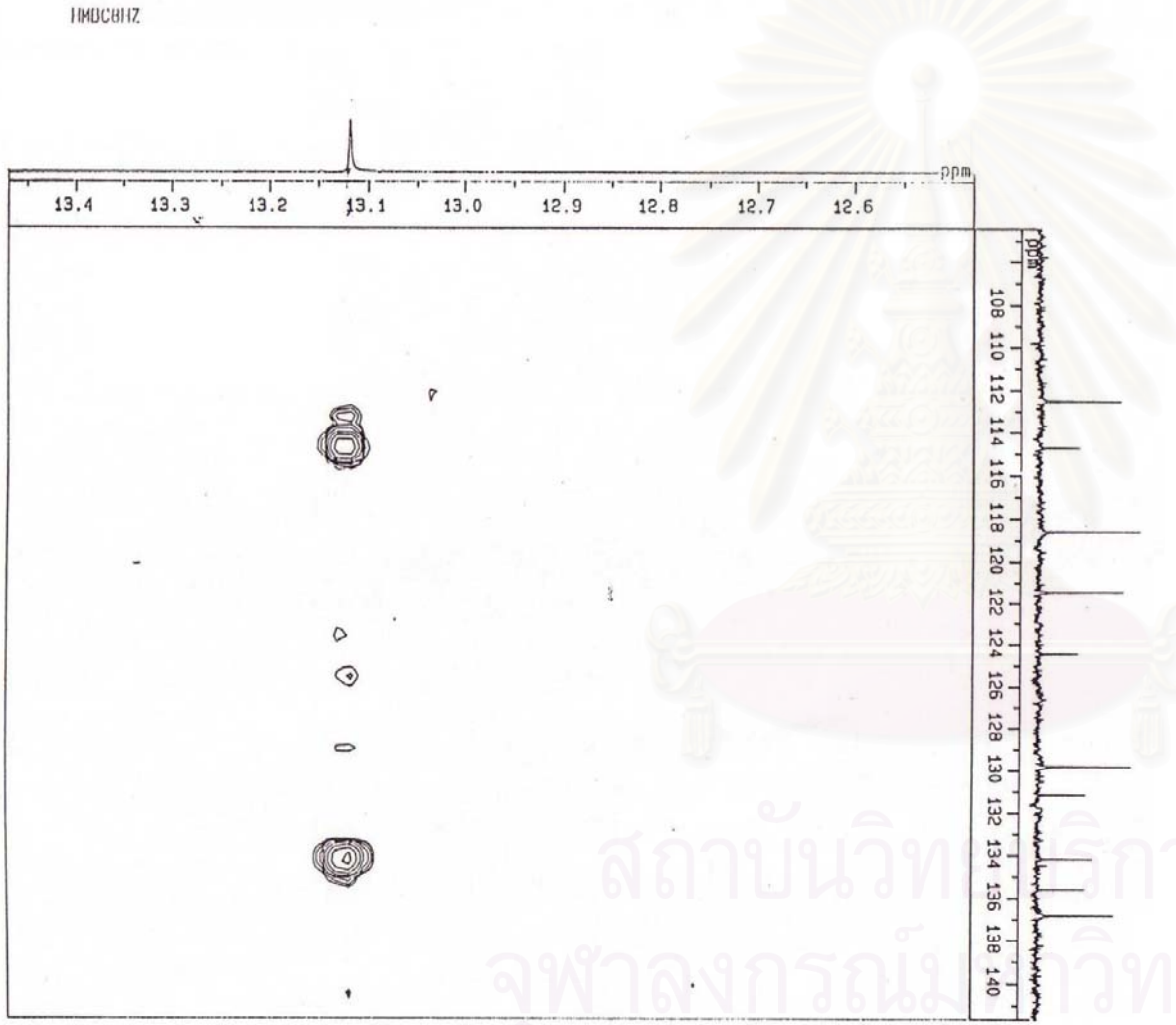


Figure 22f. The HMBC spectrum of Compound H-3 in DMSO-d_6



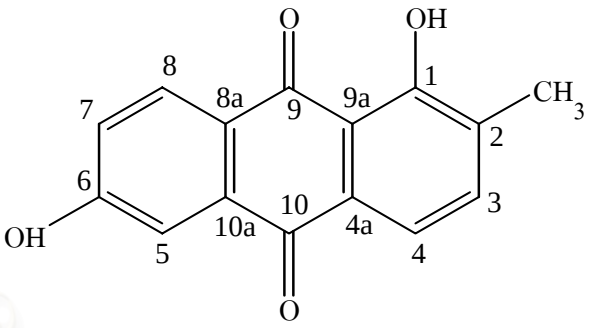


Figure 22g. The HMBC spectrum of Compound H-3 in DMSO-d_6

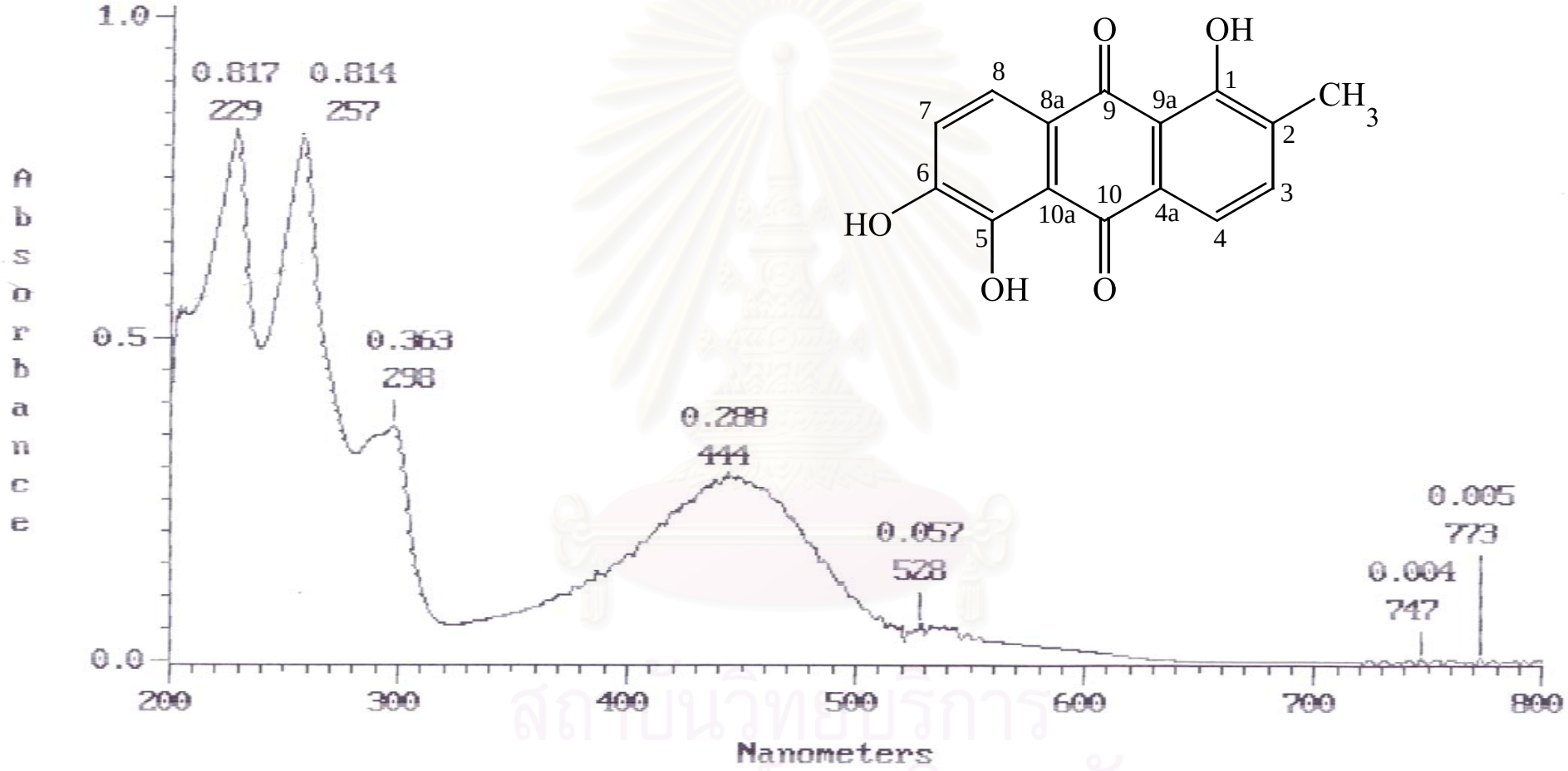


Figure 23. Ultraviolet absorption spectrum of Compound H-4 (in MeOH)

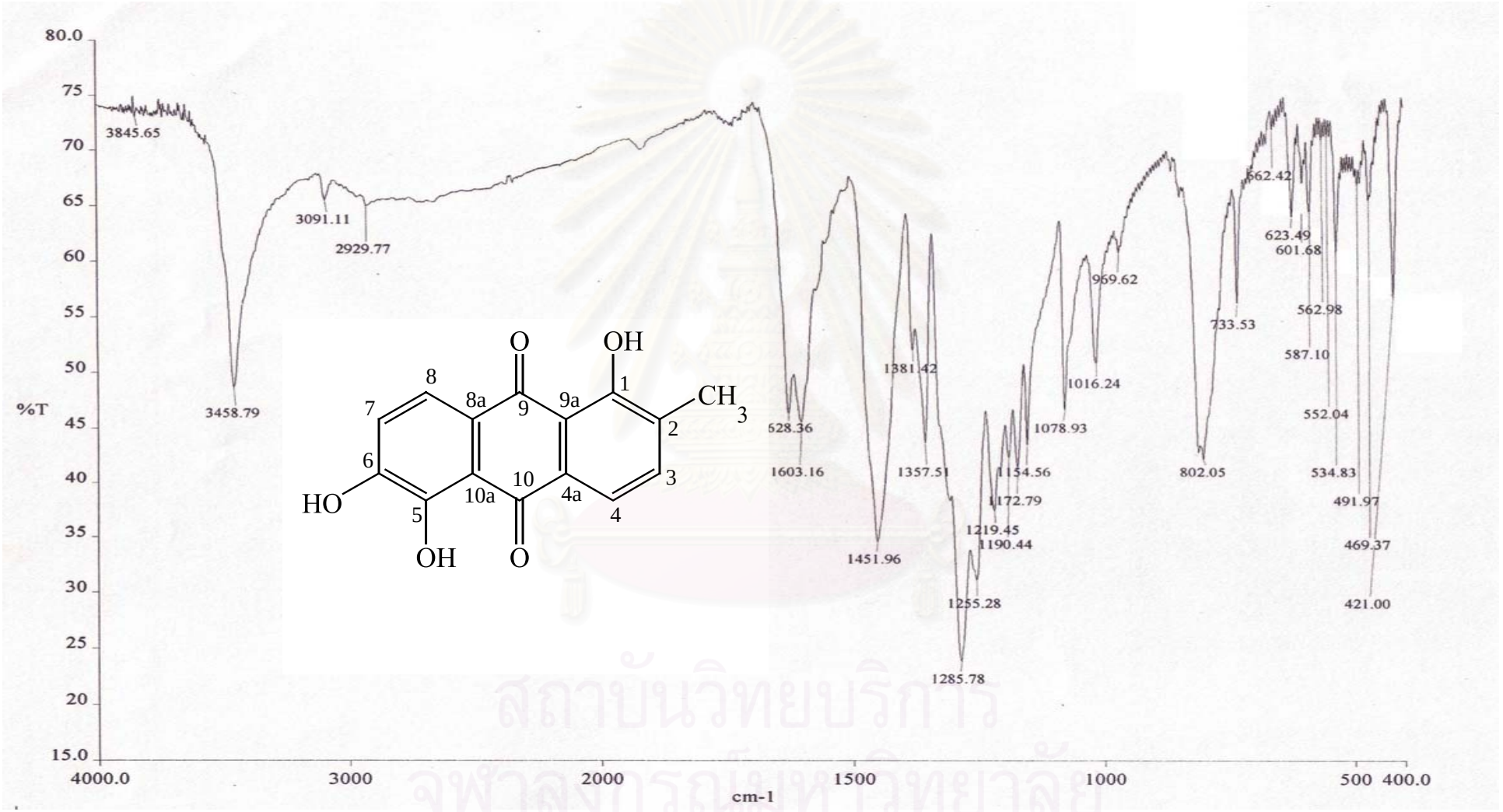


Figure 24. Infrared absorption spectrum of Compound H-4 (KBr)

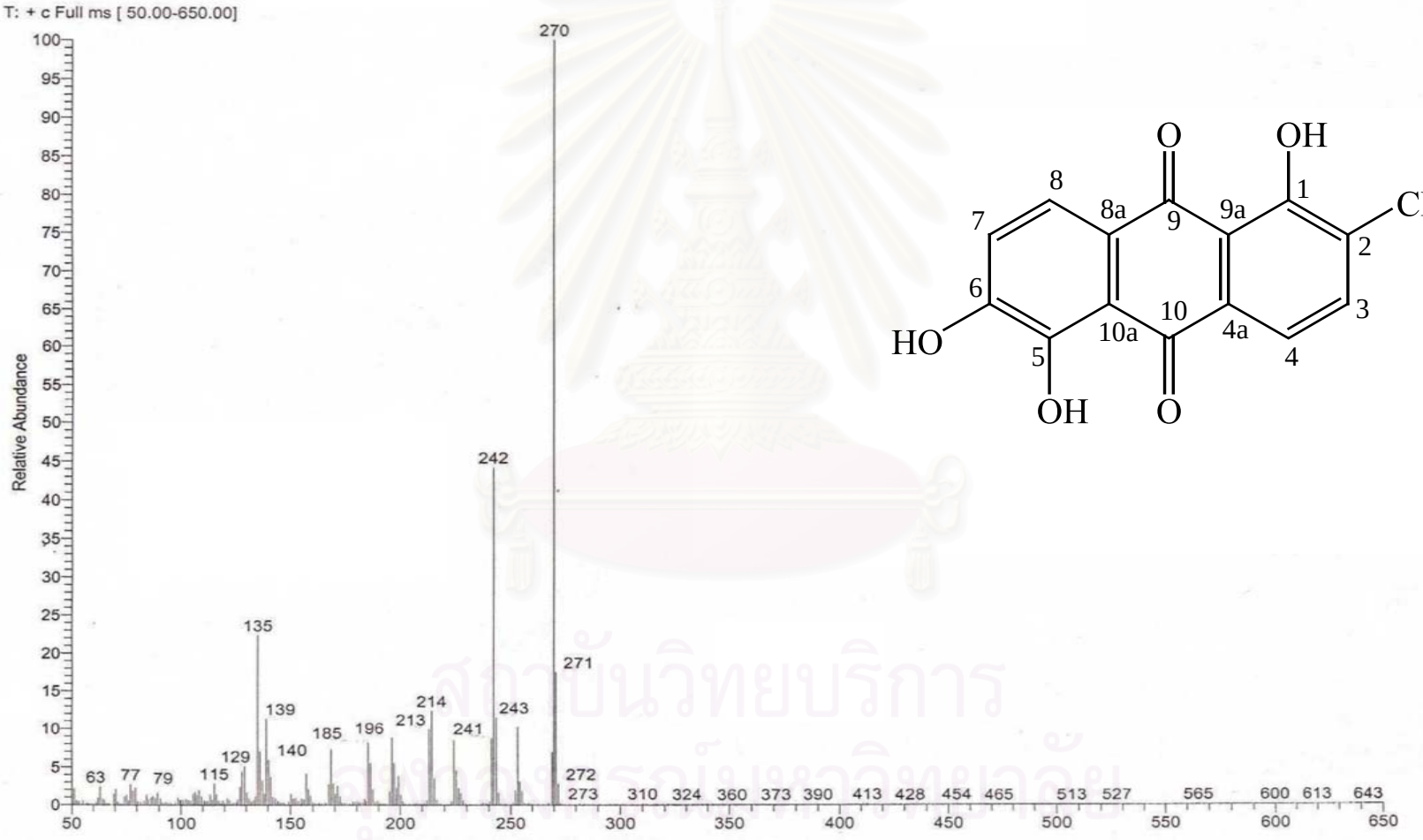


Figure 25. EI Mass spectrum of Compound H-4

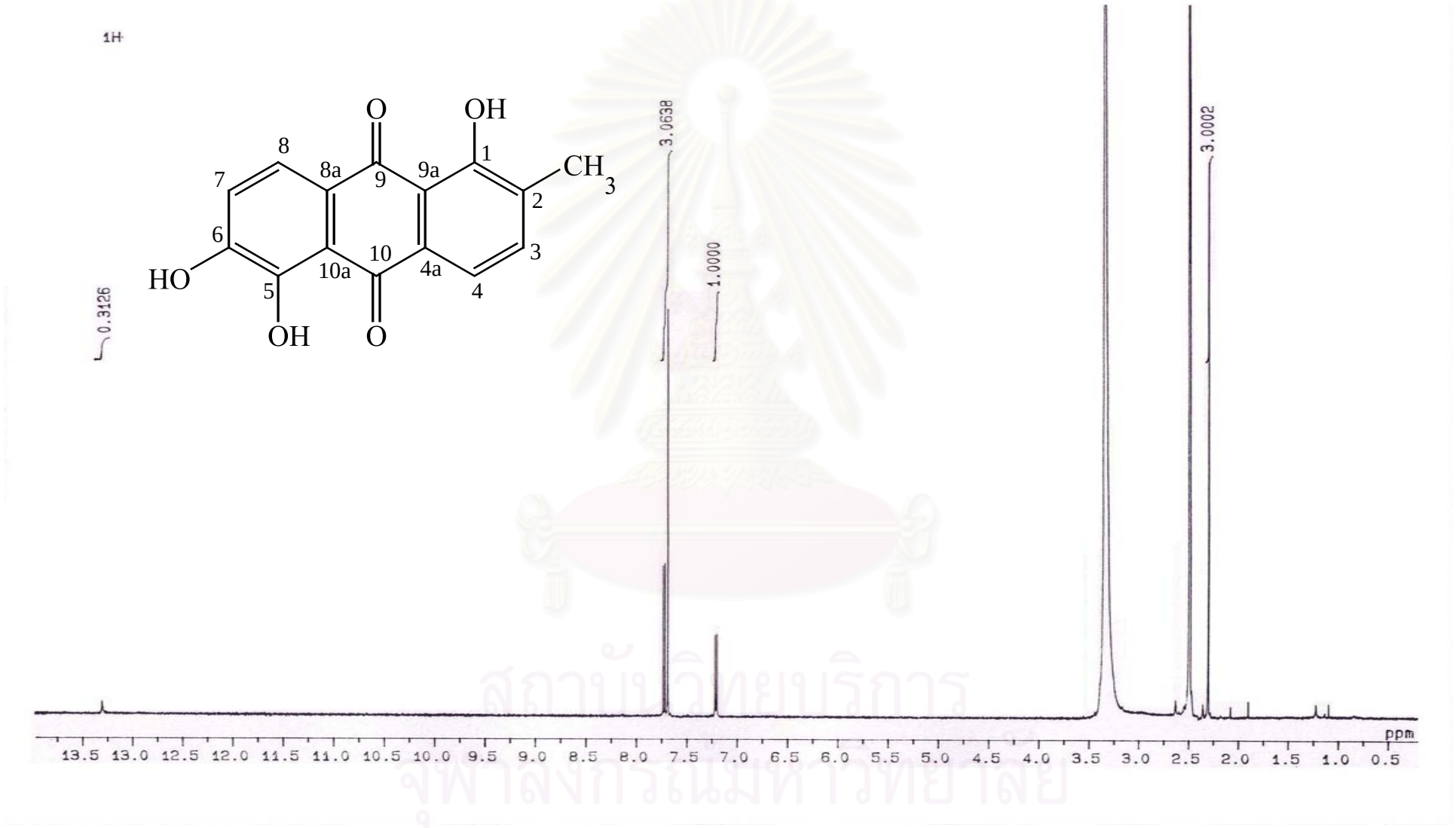
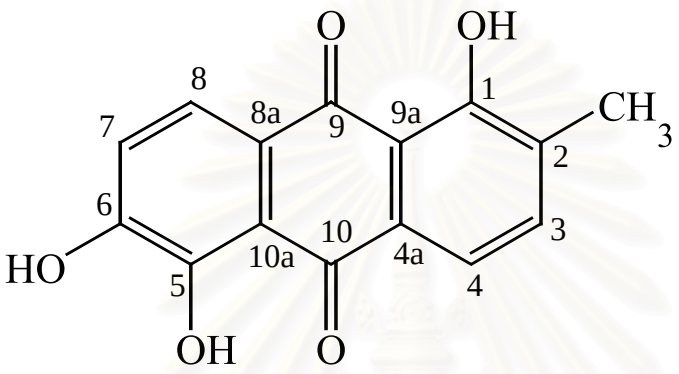


Figure 26a. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound H-4 in DMSO-d_6



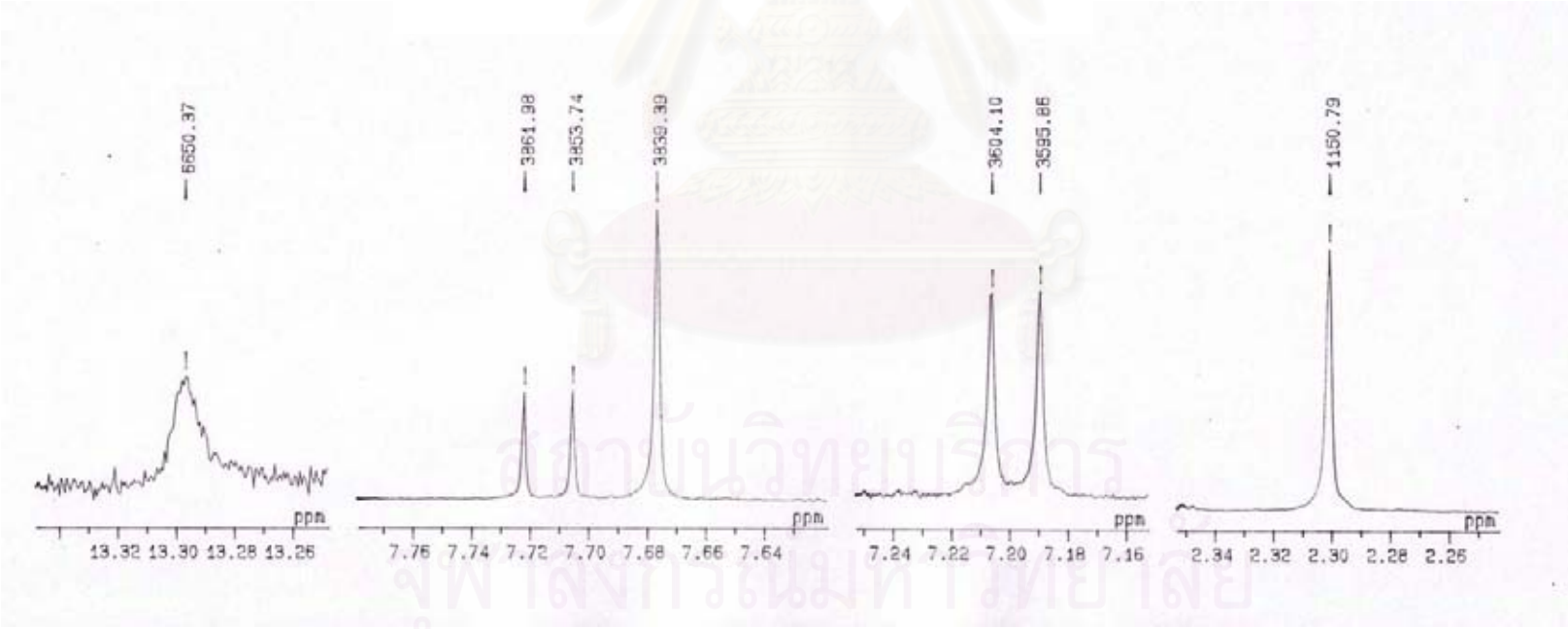


Figure 26b. The ^{1}H Nuclear magnetic resonance spectrum (500 MHz) of Compound H-4 in DMSO-d_6 (expanded)

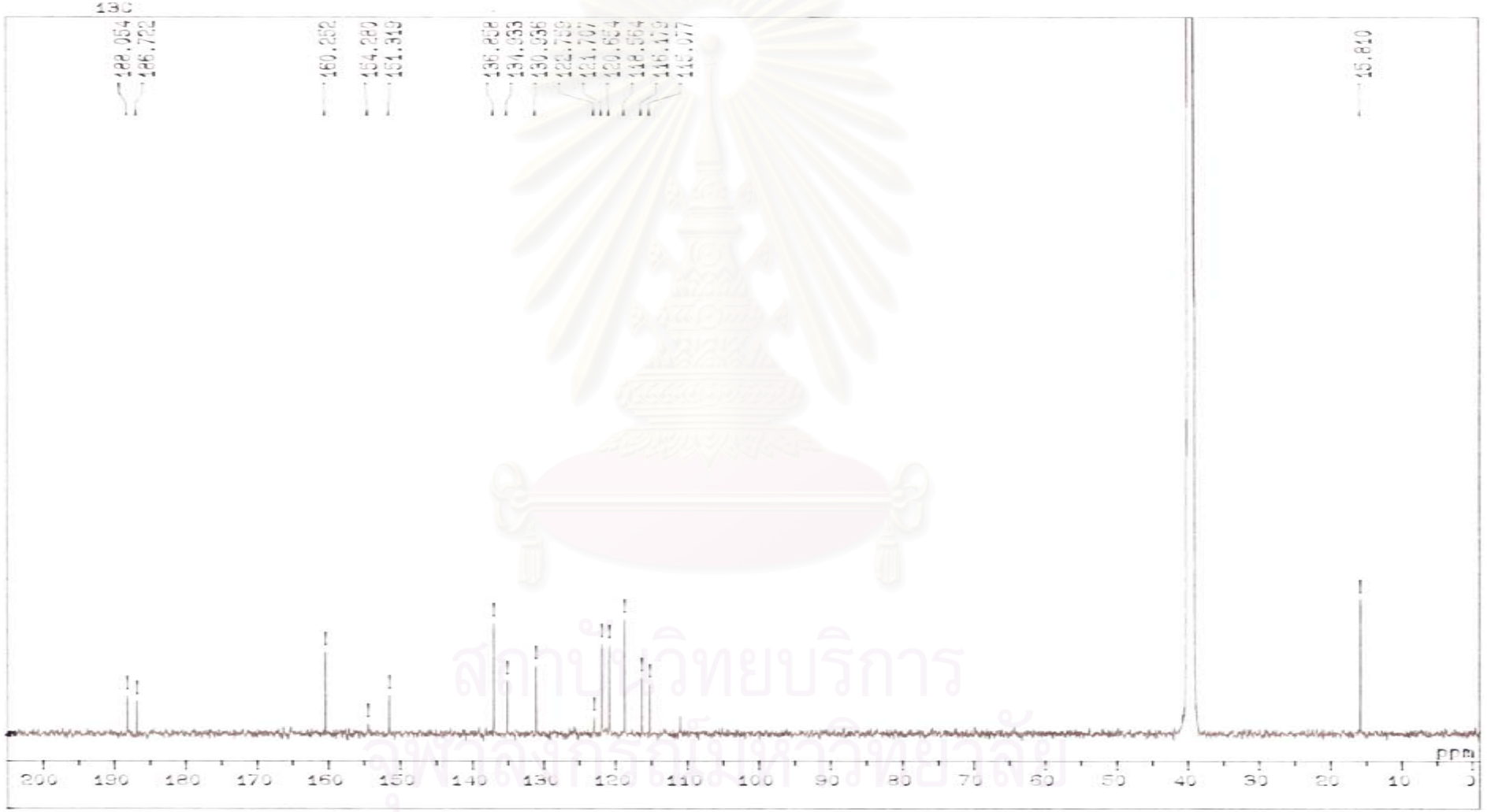


Figure 27. The ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound H-4 in DMSO-d_6

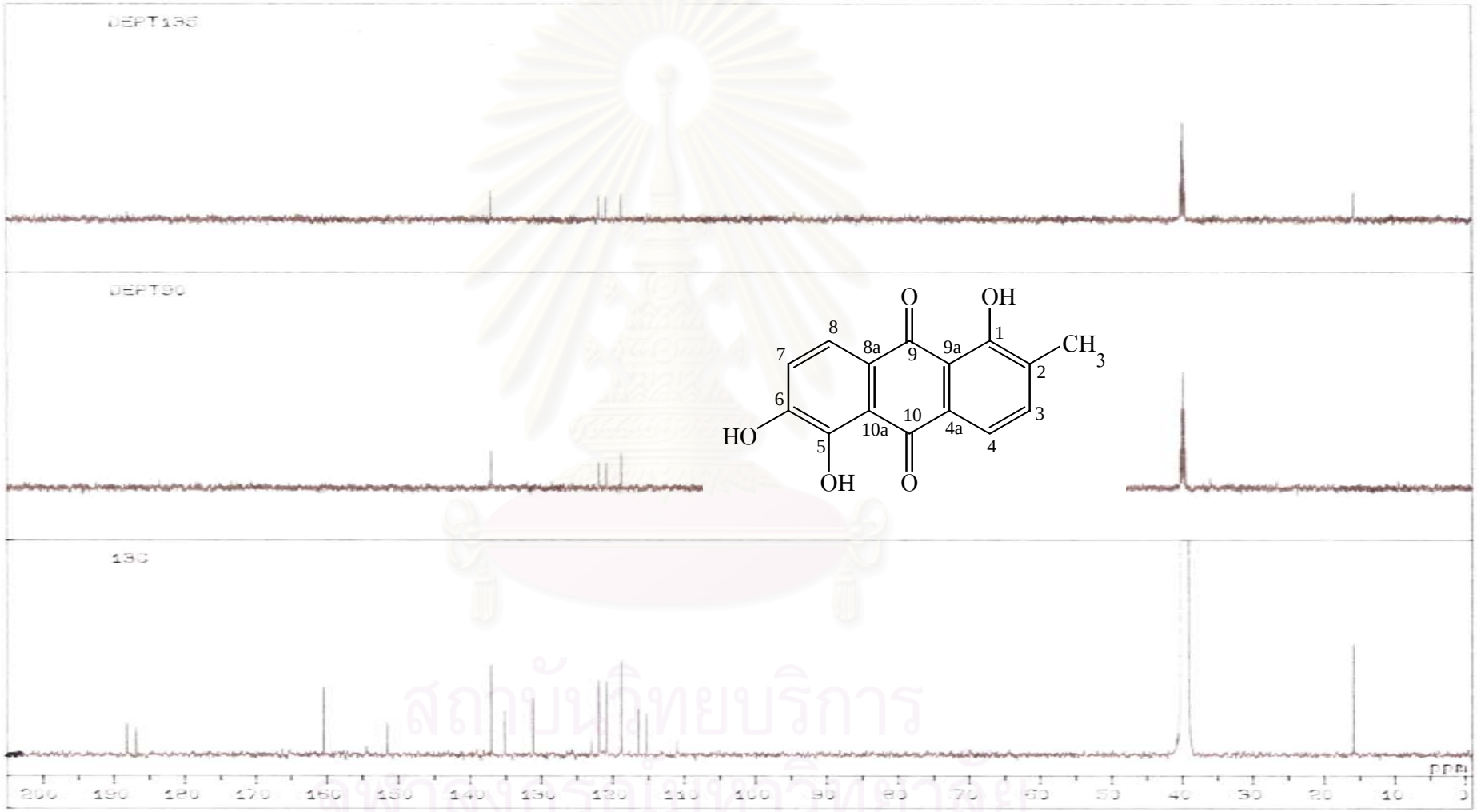


Figure 28a. The DEPT-135, 90 and ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound H-4 in DMSO-d_6

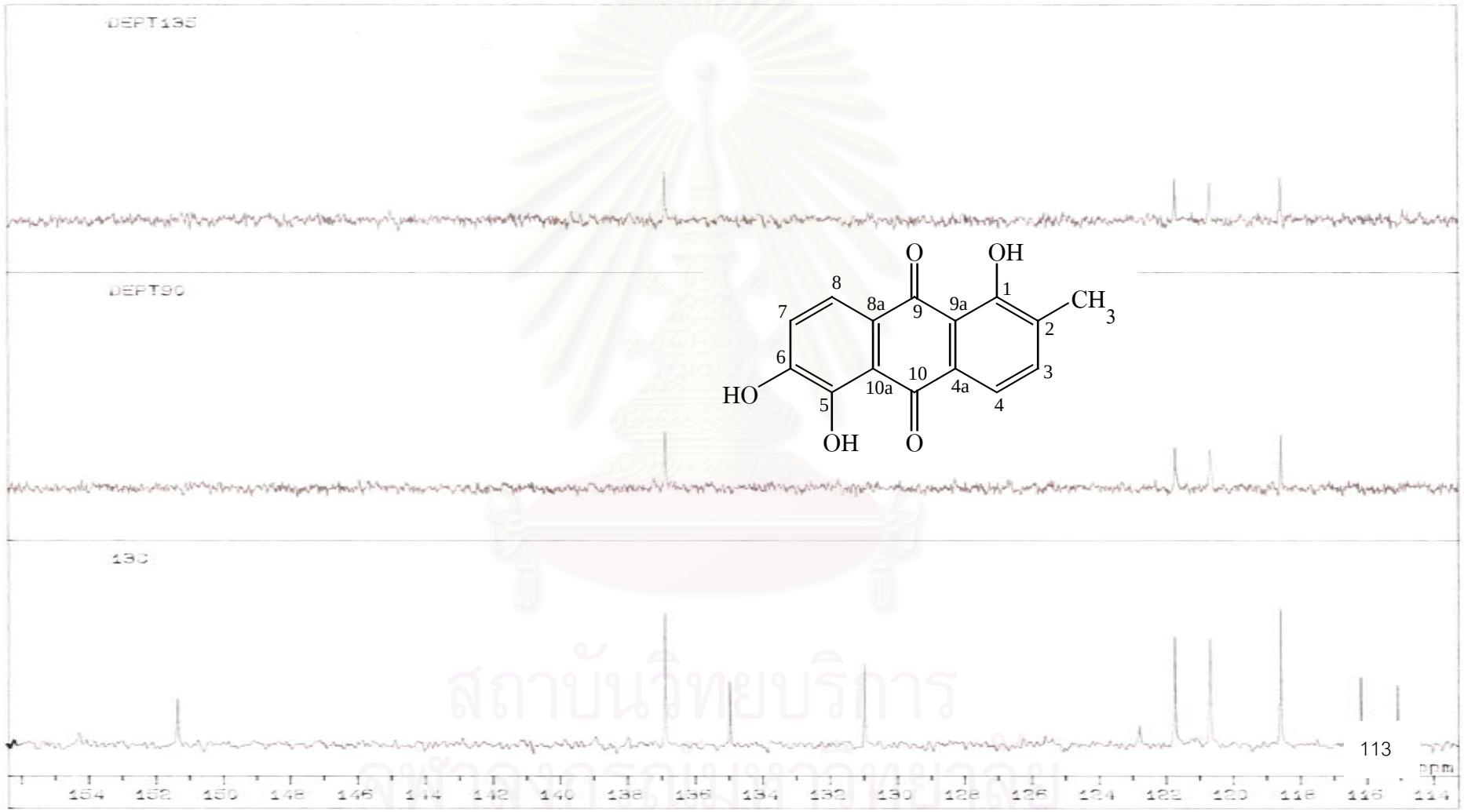
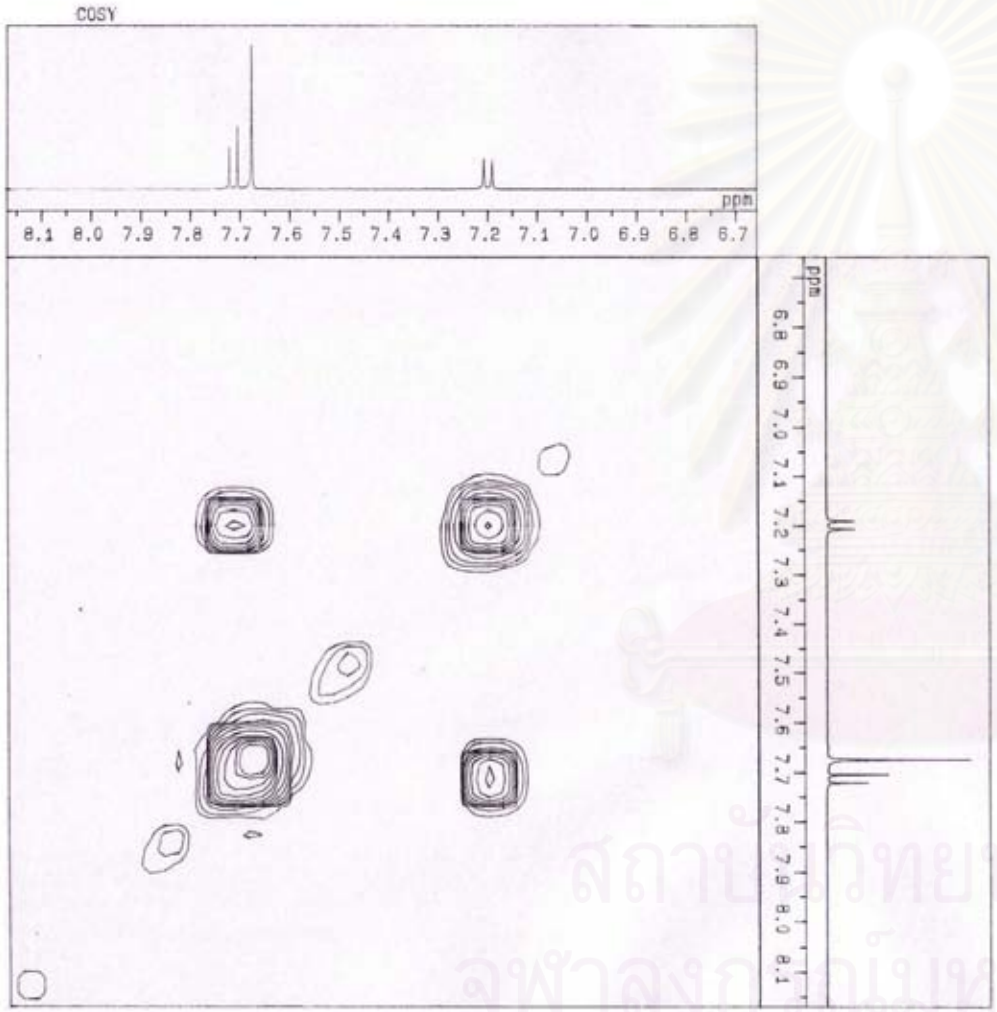


Figure 28b. The DEPT-135, 90 and ^{13}C Nuclear magnetic resonance spectrum (125 MHz) of Compound H-4 in DMSO-d_6 (expanded)



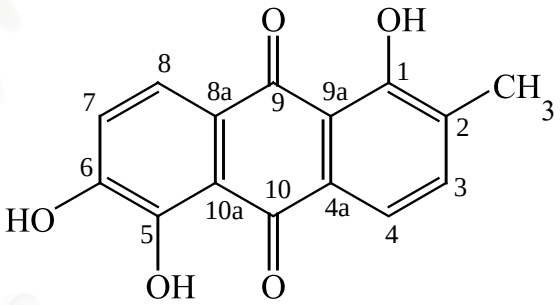
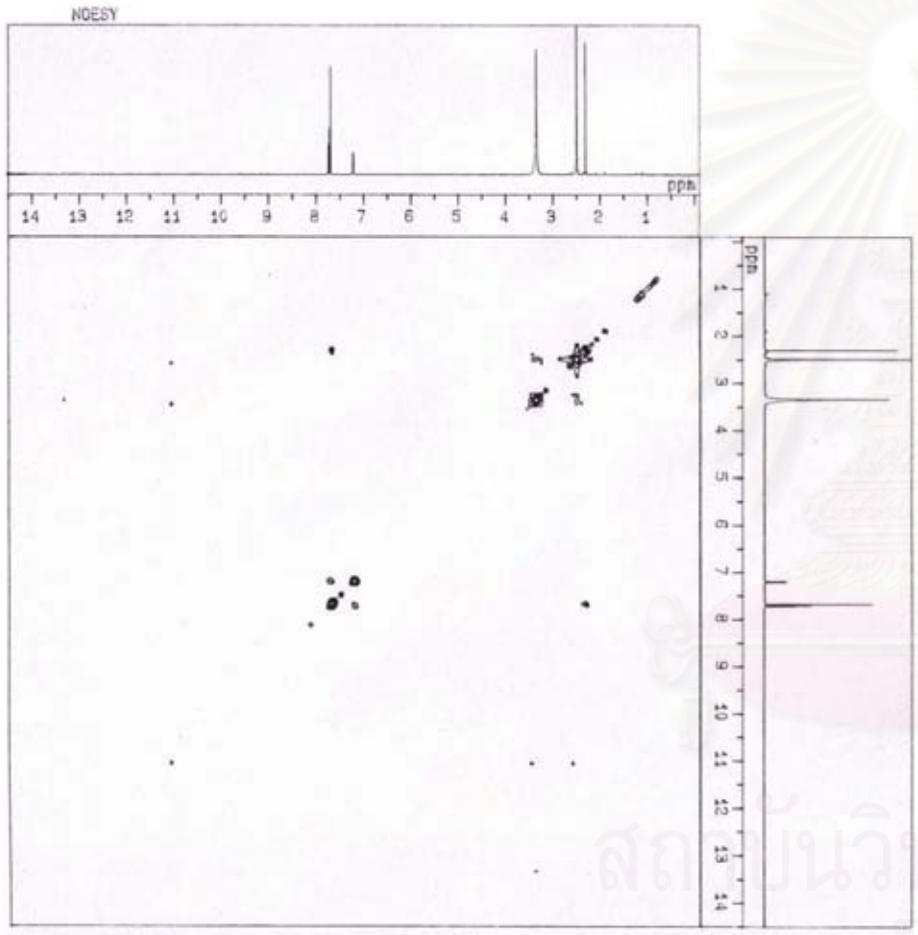


Figure 29. The ^{1}H-^{1}H COSY spectrum of Compound H-4 in DMSO-d_6



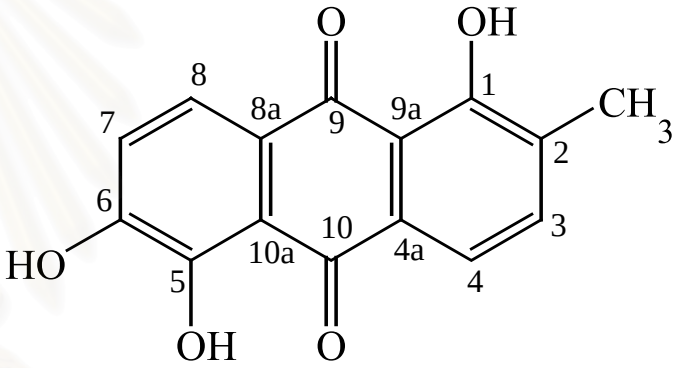
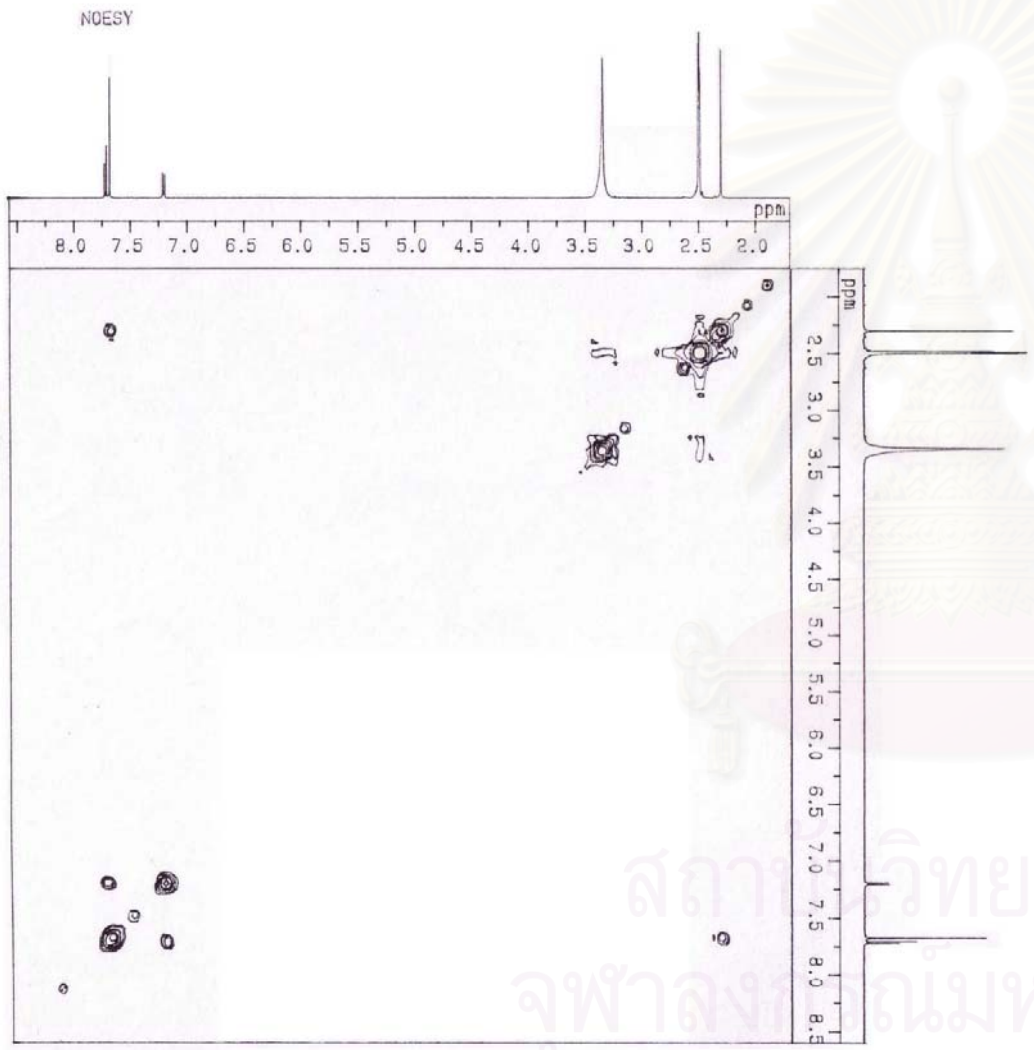


Figure 30a. The NOESY spectrum of Compound H-4 in DMSO-d_6



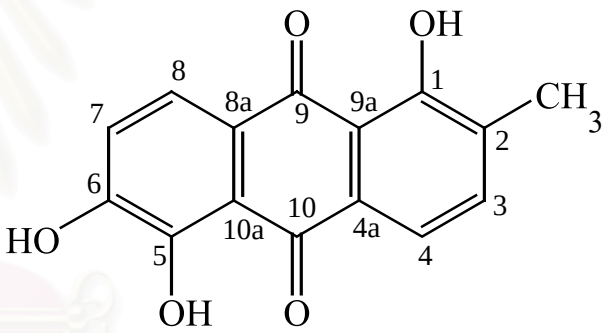
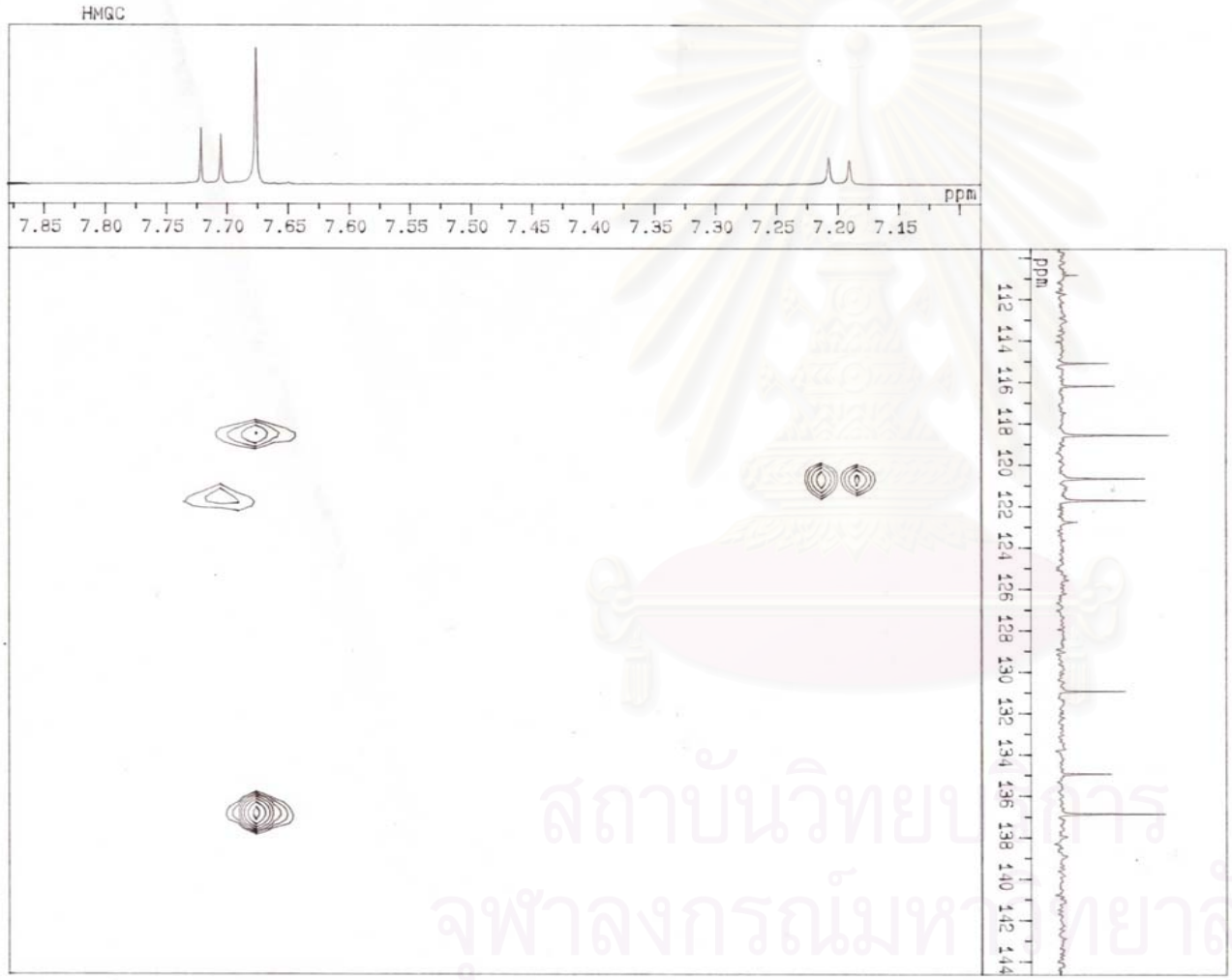


Figure 30b. The NOESY spectrum of Compound H-4 in DMSO-d_6 (expanded)



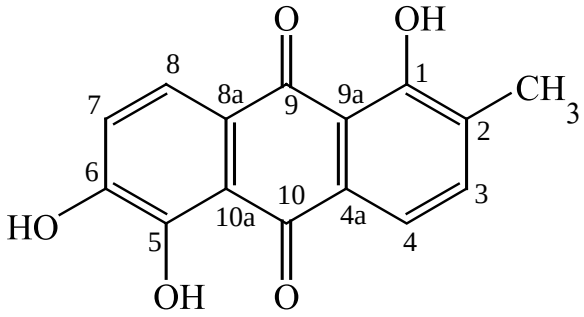
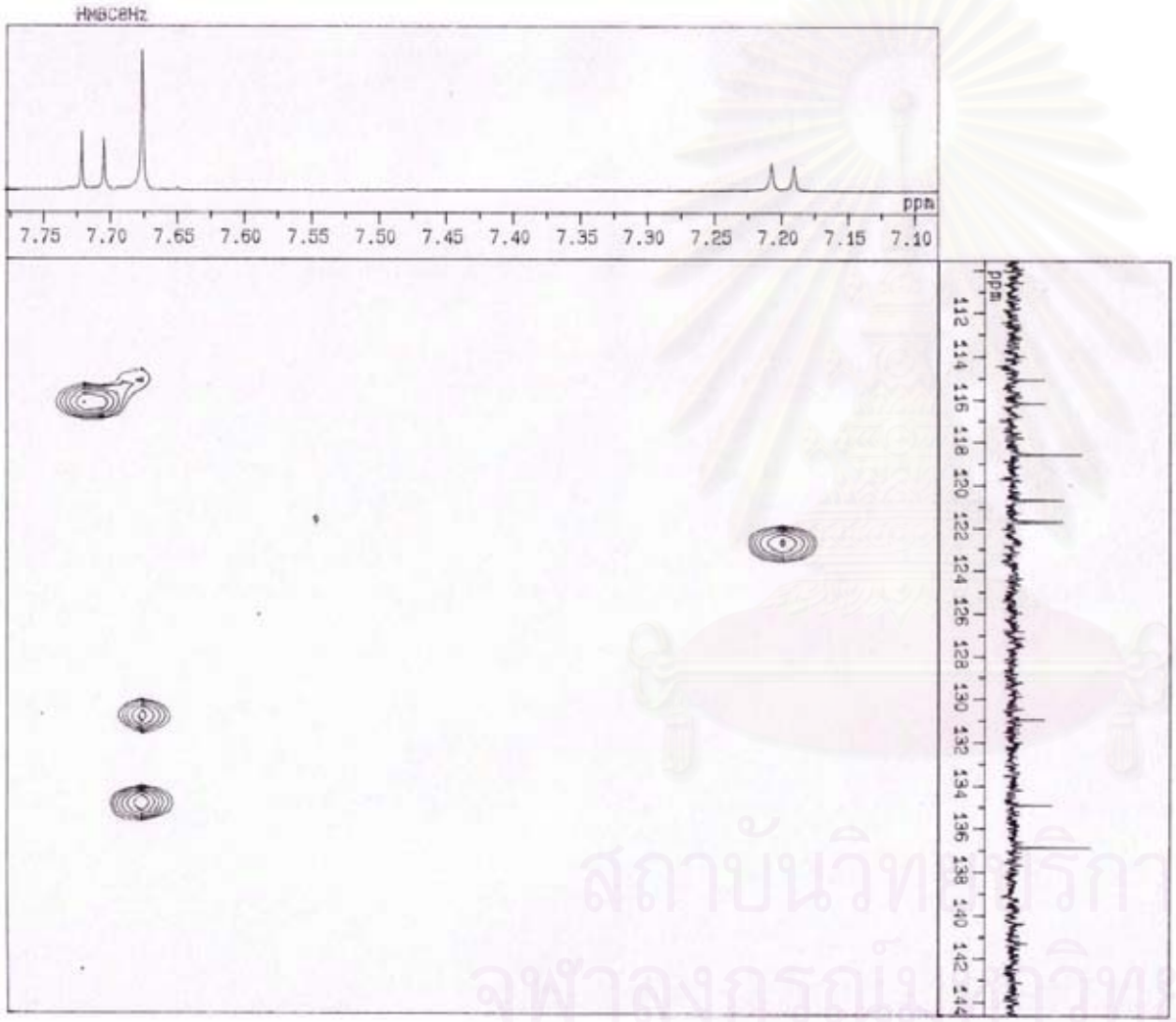


Figure 31. The HMQC spectrum of Compound H-4 in DMSO-d_6



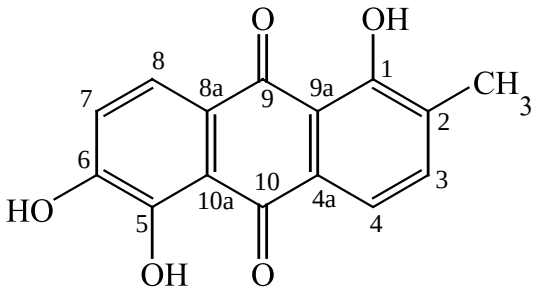
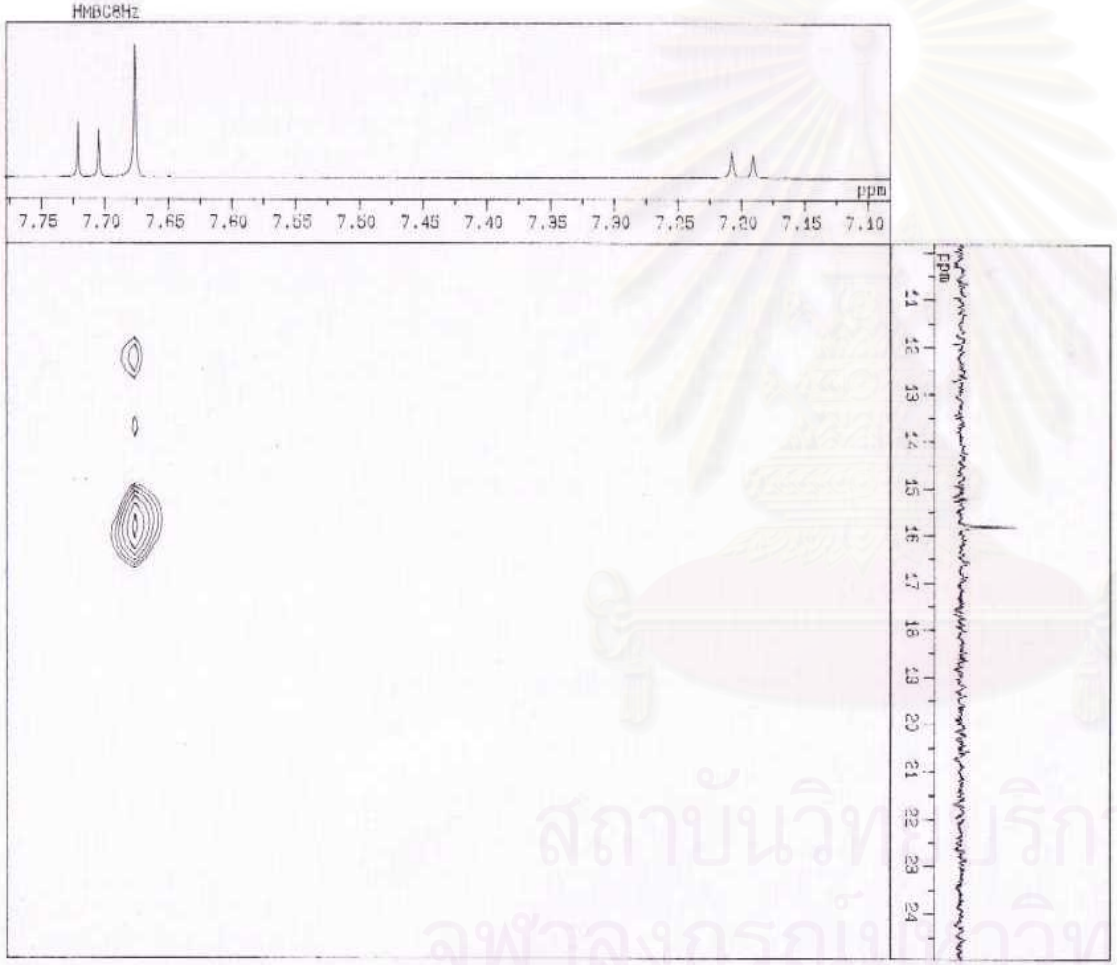


Figure 32a. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)



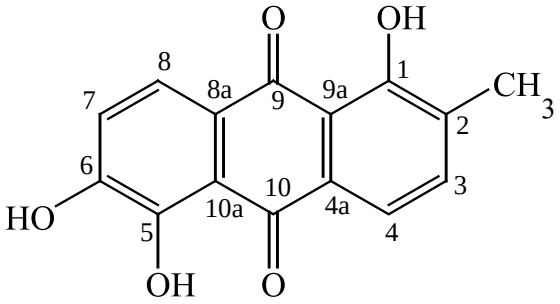
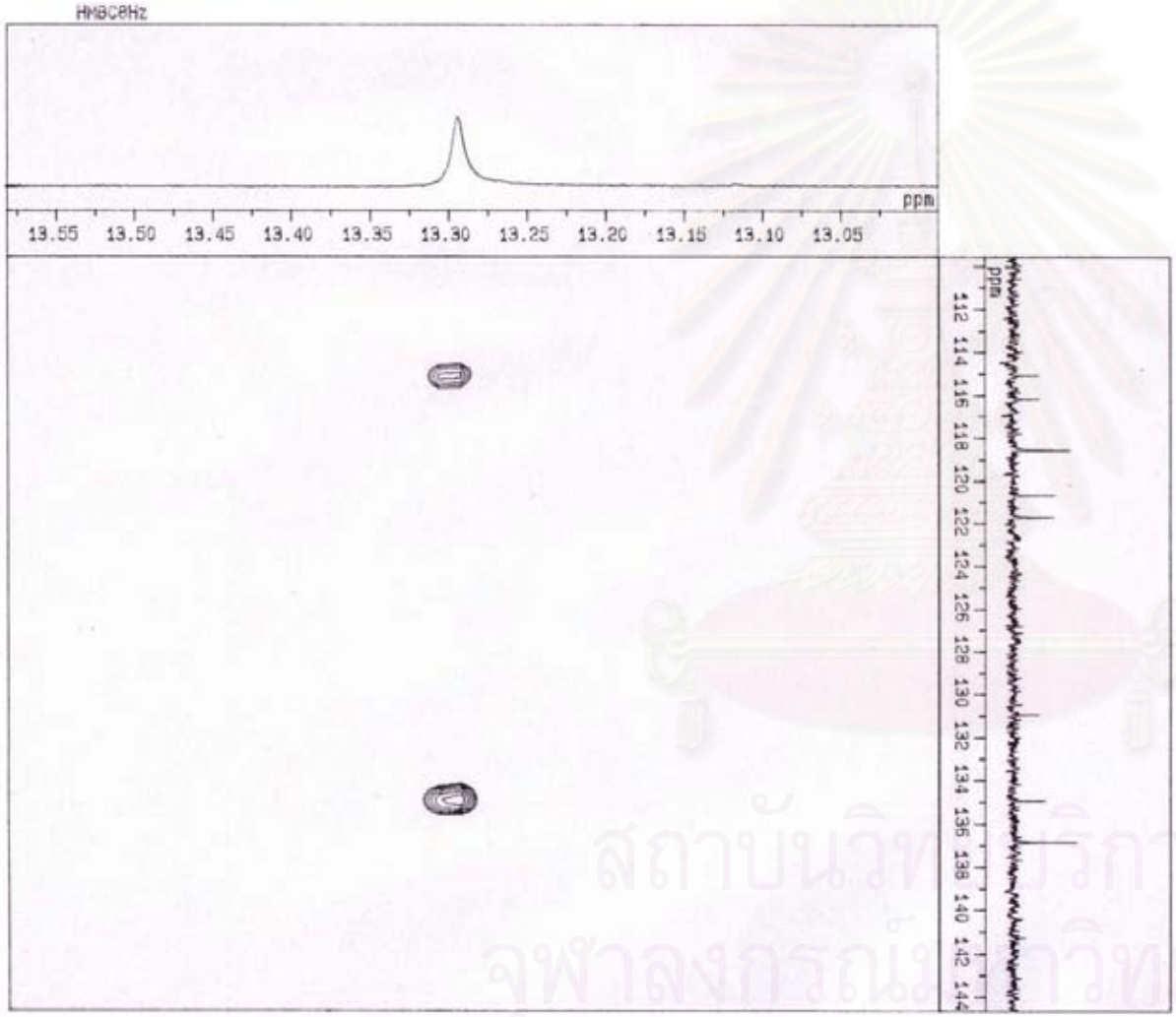


Figure 32b. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)



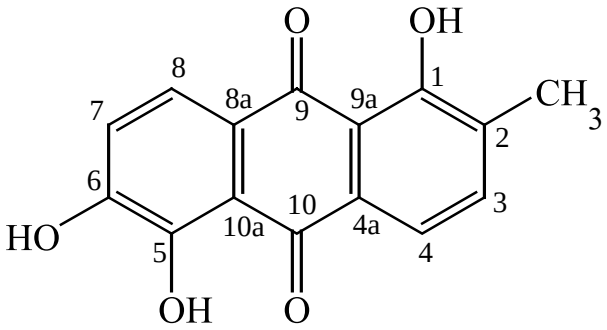
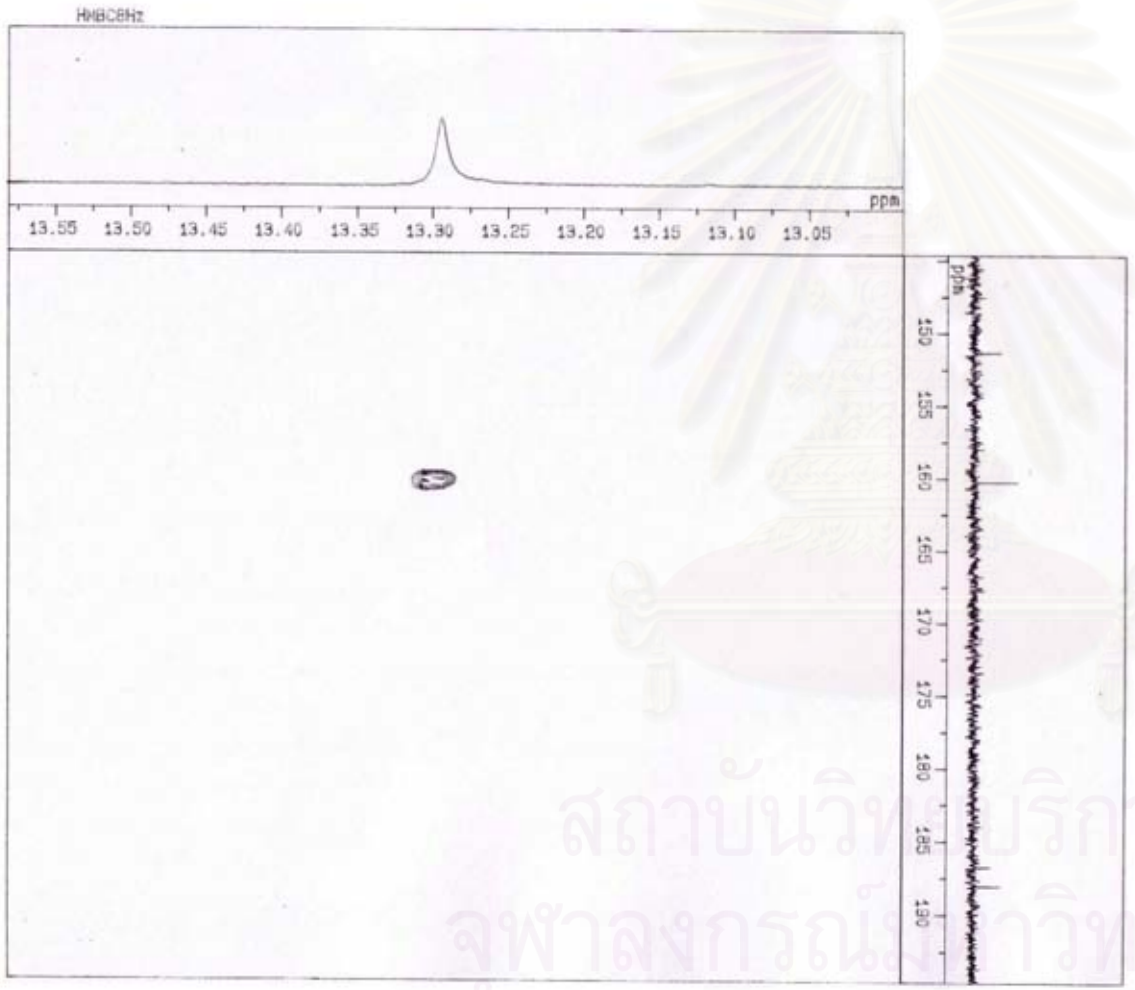


Figure 32c. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)



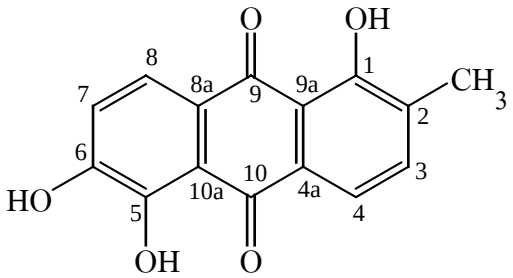
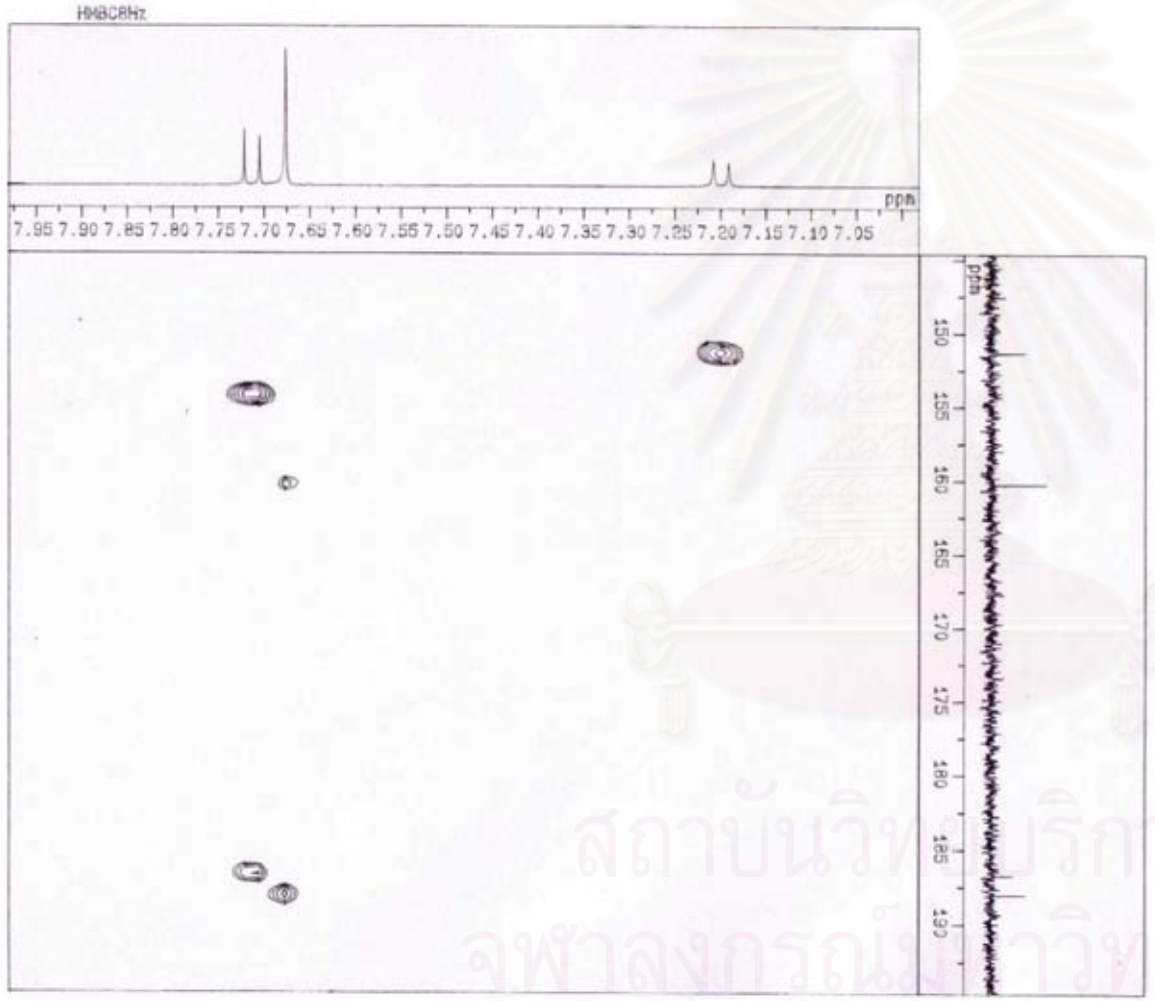


Figure 32d. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)



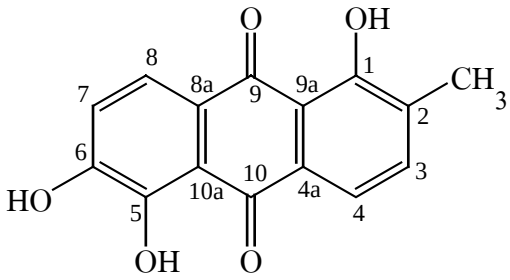
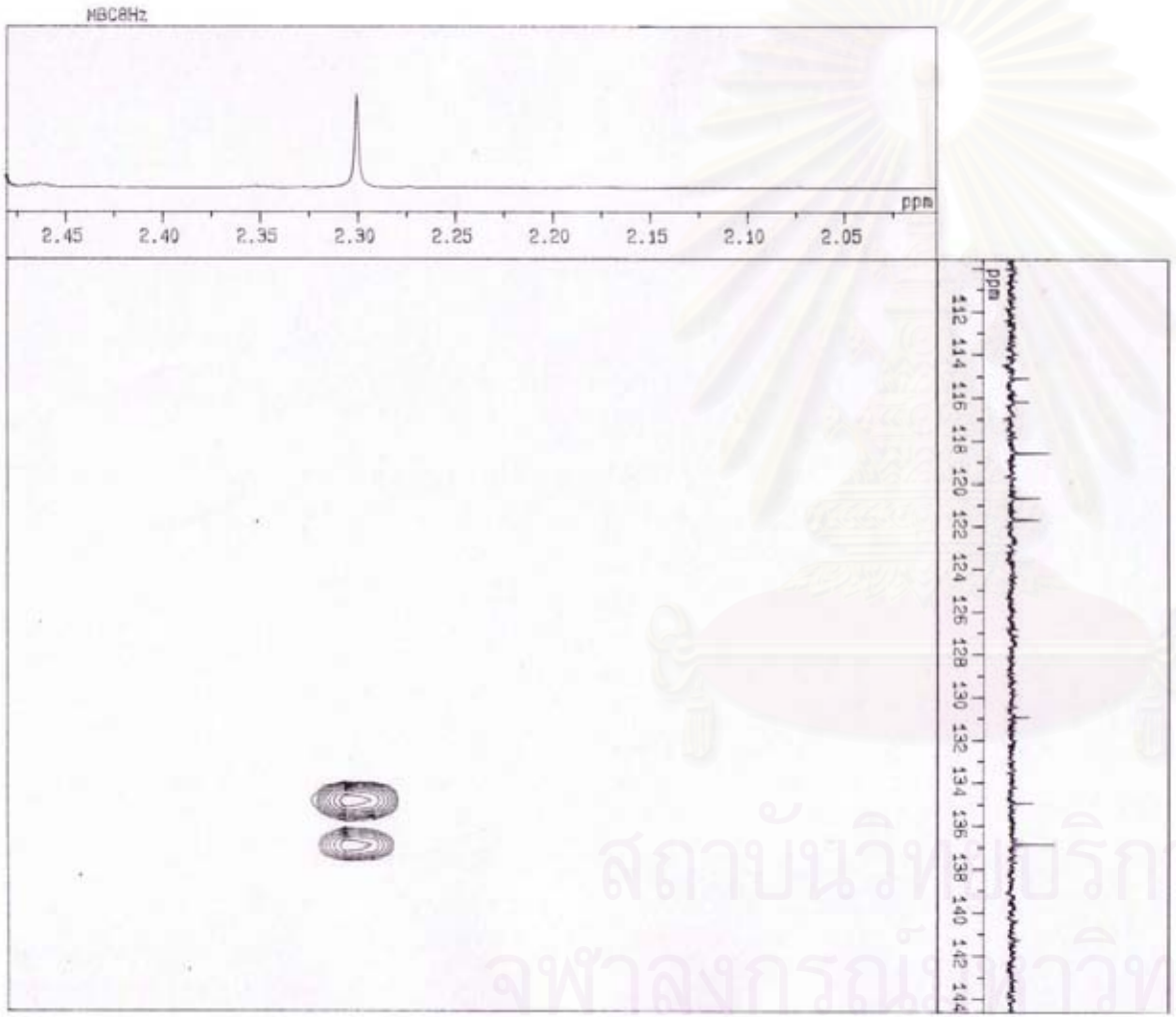


Figure 32e. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)



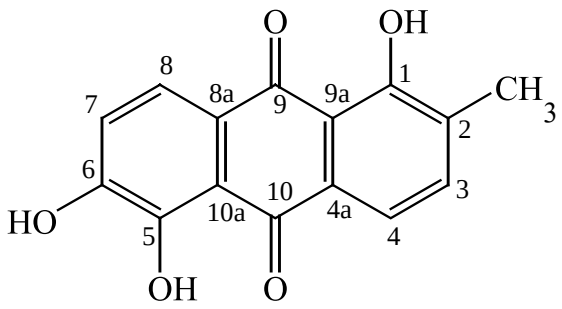
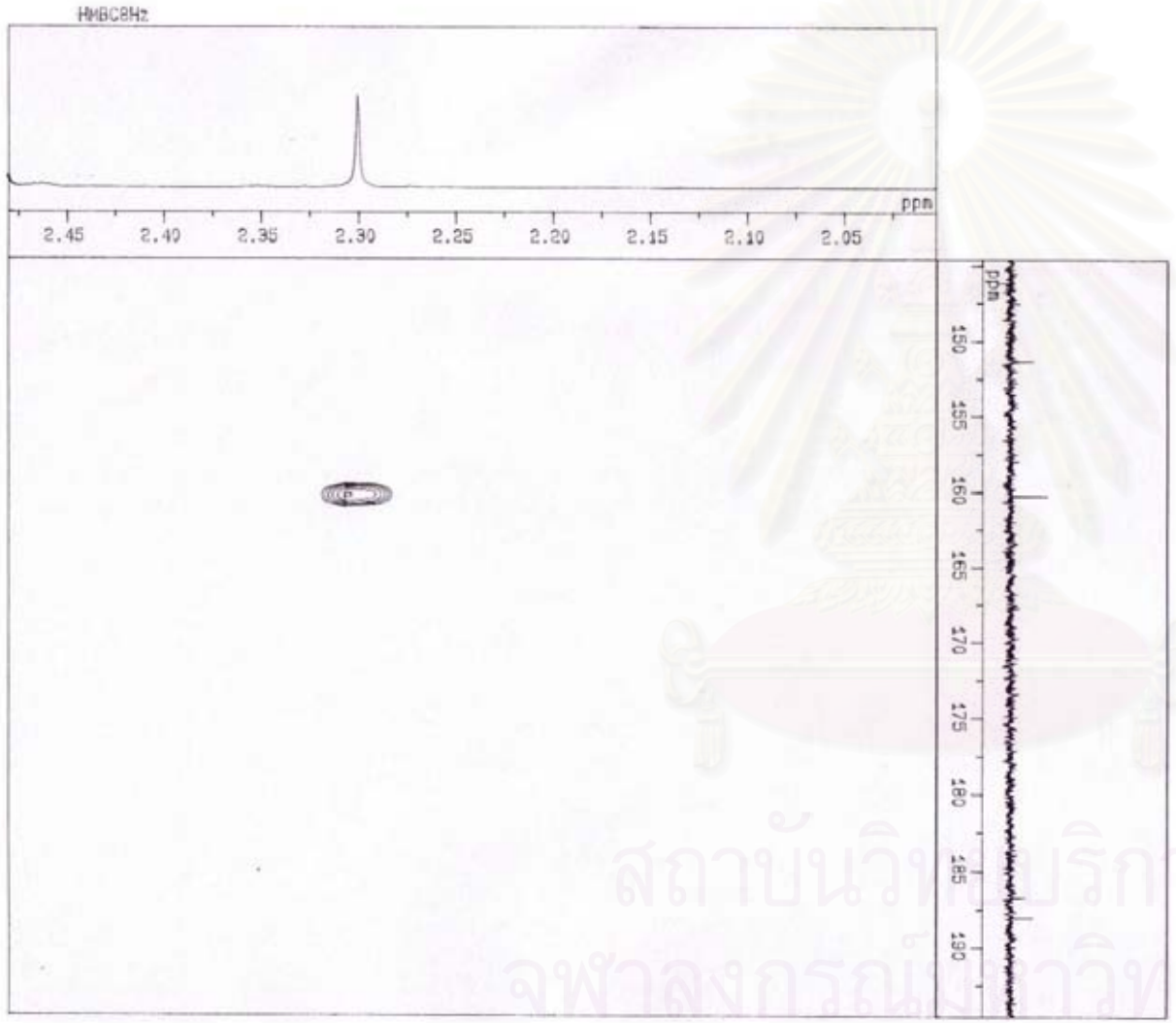


Figure 32f. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)



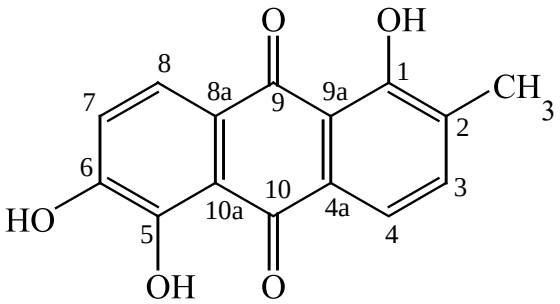


Figure 32g. The HMBC spectrum of Compound H-4 in DMSO-d_6 (expanded)

VITA

Miss Patchara Booranachad was born on May 3rd, 1974 in Bangkok, Thailand. She obtained a Bachelor's degree of Science in Pharmacy in 1997 from the Faculty of Pharmaceutical Sciences, Prince of Songkhla University, Thailand. Since her graduation, she has been working as a pharmacist at Department of Pharmacy, Thammasat Hospital, Faculty of Medicine, Thammasat University, Thailand.