องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของเปลือกต้นเปล้าใหญ่ (*Croton oblongifolius* Roxb.) จากอำเภอวังสะพุง จังหวัดเลย

นางสาวนฤภัทร คุปติยานุวัฒน์

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CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY FROM STEM BARKS OF *Croton oblongifolius* Roxb. FROM AMPHOE WANG SAPHUNG, LOEI PROVINCE

Miss Narupat Kuptiyanuwat

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry

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(Associate Professor Chaiyo Chaichantipyuth, M.Phar.)

นฤภัทร คุปติยานุวัฒน์ : องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของเปลือกต้นเปล้าใหญ่ (*Croton oblongifolius* Roxb.) จากอำเภอวังสะพุง จังหวัดเลย (CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY FROM STEM BARKS OF *Croton oblongifolius* Roxb. FROM AMPHOE WANG SAPHUNG, LOEI PROVINCE อาจารย์ที่ปรึกษา : รศ. ดร. อมร เพชรสม 99 หน้า. ISBN 974-334-014-9

ได้มีการสกัดแยกสารประกอบแลบเดนไดเทอร์ปีนอยด์ใหม่สามชนิดคือ 2-acetoxy-labda-8(17),12(*E*),14-triene-3-ol (1), 3-acetoxy-labda-8(17),12(*E*),14-triene-2-ol (2), labda-8(17),12(*E*),14-triene-2,3-diol (3) จากเปลือกต้นเปล้าใหญ่ อำเภอวังสะพุง จังหวัดเลย และได้ทำการพิสูจน์สูตรโครงสร้างของสารใหม่นี้โดยอาศัยข้อมูลทางสเปกโตรสโกปี ซึ่งได้แก่ IR, MS, 1D และ 2D NMR เทคนิคคือ DEPT, COSY, NOESY, HMBC และ HMQC และนำมาทดสอบการยับยั้งเซลล์มะเร็งในหลอดทดลองกับเซลล์มะเร็ง KATO-3 (กระเพาะอาหาร), SW 620 (ลำไส้), BT474 (เต้านม), HEP-G2 (ตับ) และ CHAGO (ปอด) พบว่า สาร 3 มีฤทธิ์ยับยั้งเซลล์มะเร็งทั้ง 5 ชนิด สาร 1 มีฤทธิ์ยับยั้งเซลล์มะเร็งชนิด KATO-3 (กระเพาะอาหาร) และ SW 620 (ลำไส้) ส่วนสาร 2 มีฤทธิ์ยับยั้งเซลล์มะเร็งชนิด KATO-3 (กระเพาะอาหาร) และ BT 474 (เต้านม)

ภาควิชา.....เคมี..... ลายมือชื่อนิสิต.....นฤภัทร คุปติยานุวัฒน์.....

สาขาวิชา..... เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....อมร เพชรสม.....

ปีการศึกษา.....2542.....

NARUPAT KUPTIYANUWAT: CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY FROM THE STEM BARKS OF *Croton oblongifolius* Roxb. FROM AMPHOE WANG SAPHUNG, LOEI PROVINCE THESIS ADVISOR : ASSO. PROF. AMORN PETSOM, Ph.D. 99 pp. ISBN 974-334-014-9

Three new labdane diterpenoid compounds, 2-acetoxy-labda-8(17),12(*E*),14-triene-3-ol (1), 3-acetoxy-labda-8(17),12(*E*),14-triene-2-ol (2), labda-8(17),12(*E*),14-triene-2,3-diol (3) were isolated from the stem bark of *Croton oblongifolius* Roxb.. The structure of the new compounds were established by spectroscopic data (IR, MS spectra, 1D and 2D NMR techniques including DEPT, COSY, NOESY, HMBC and HMQC) and they were tesed for cytotoxicity against various human tumor cell lines (KATO-3 (gastric), SW 620 (colon), BT474 (breast), HEP-G2 (hepatoma) and CHAGO (lung)). Compound 3 showed moderate activities against KATO-3 (gastric), SW 620 (colon), BT474 (breast), HEP-G2 (hepatoma) and CHAGO (lung)cell line. Compound 1 was active against KATO-3 cell line and SW 620 cell line. Compound 2 was active against KATO-3 (gastric) and BT 474 (breast) cell line.

ภาควิชา.....เคมี..... ลายมือชื่อนิสิต.....นฤภัทร อุปติยานุวัฒน์.....

สาขาวิชา..... เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา....2542.....

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Deep and sincere gratitude was acknowledged to her parents, her sister and her friends for their understanding and encouragement throughout the entire study.

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Fig.	Figure
cm.	centrimeter
mm.	millimeter
wt	weight
eV.	electron Volt
MHz	MegaHertz
TLC	Thin Layer Chromatography
kg	kilogram (s)
g	gram (s)
cm^3	cubic of centimeter
$CHCl_3$	chloroform
MeOH	methanol
EtOAc	ethyl acetate
no.	number
mg	miligram
mp	melting point
KBr	potassium bromide
ν_{max}	the wavenumber at maximum absorption
cm^{-1}	unit of wavenumber
s	strong (IR)
m	medium (IR)
w	weak (IR)
temp.	temparature
^oC	degree celsius
ml	milliliter (s)
min	minute
R_f	rate of flow in chromatography

$CDCl_3$	rate of flow in chromatography
δ	chemical shift
ppm	part per million
m/e	mass to charge ratio
DEPT	Distortionless Enhancement by Polarisation Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
COSY	Correlated Spectroscopy
NOESY	Neclear Overhauser Enhancement Spectroscopy

CHAPTER I

INTRODUCTION

Medicinal plants are natural products that can be used as therapeutic agent. They are cheap, effective and have less harmful side effects than synthetic drugs [1]. Accordingly, medicinal plants are wildly studies by modern techniques in a more scientific way. There has also been an effort to develop new drugs from medicinal plants in order to make them safe and effective drugs. From the Thai Ethropharmacophae, Plao-yai (*Croton oblongifolius* Roxb.) is often used with plao-noi, (*Croton sublyratus* Kurz.) as antipeptic ulcer drug. Therefore, its is very interesting to investigate chemical constituents of plao-yai for effective "lead" compound.

Plao-yai (*Croton oblongifolius* Roxb.) belongs to the Euphorbiaceae family[2]. It is found in evergreen forests, deciduous forests and groves of brushwood, which is not more than 700 meters above sea level [3]. In Thailand, has several local names including "Plao-yai" in Central Plao Luang (Northern), Khwa-wuu (Karen in Kanchanaburi), Seng-khe-khang, Sa-kaa-waa and Saa-kuu-wa (Karen in Mae Hong Son), Poh (Kamphaeng Phet) and Haa-yoeng (Shan in Mae Hong Son)[3].

Plao-yai is a very interesting medicinal plant because all parts of plao-yai have been used in Folk medicine [3,4,5,6]. The barks are used to inhibit chronic enlargements of livers, leaves can be used as a tonic, fruits are used to treat dysmenorrhea, seed are used as a purgative, flowers are used to kill parasite, heartwood is remedy of faint and roots are remedy for dysentery.

General Characterization of the Plants in the Genus Croton [7]

The genus Croton comprises of 700 species of trees or shrubs. Leaves are usually alternate with 2-glandular stipule at the base. Their flowers are solitary or clustered in the rachis of a terminal raceme and bracts are small. Male flowers contain 5-calyx, 5 petals and disk of 4-6 glands opposite the sepals. There are many stamens inserted on a hairy receptacle and anthers are adnate with parallel cells. In female flowers, sepals are usually more ovate than the male, petals are smaller than the sepals or missing and disk annular of 4-6 glands are opposite the sepals. There are three ovaries with solitary ovary in each cell. Seeds are smooth, albumen copious and broad cotyledons.

General Characterization of *Croton oblongifolius* Roxb.[3,4,5,7]

Croton oblongifolius Roxb. is a medium sized tree. Its calyx and ovary are clothed with minute orbicular silvery scales. Leaves are 5.7-11.5 by 12.5-25.0 cm in size, and crowded toward the end of the branchlets. The shape of leaf blade is oblong-lanceolate and the base is usually acute with no apparent glands above the petioles are 1.3-6.0 cm long. Flowers are pale yellowish green and solitary in the axial of minute bracts on long erect raceme. The male flowers locate in the upper parts of the raceme and the females in the lower part. Male flowers are slender and have the length of pedicels of 4.0 mm. Calyx is more than 6.0 mm long and segments are ovate, obtuse and more than 2.5 mm long. Petals are 3.0 mm long, elliptic-lanceolate and woolly. The twelve stamens are inflexed in bud and the length of filaments is 3.0 mm. In female flowers, the pedicels are short and stout. Its sepals are more acute than in the male with densely cliated margins. Petals are 2.0 mm long, with densely woolly margins. The three styles are 4.0 mm

long. Diameter of fruit is less than 1.3 cm, slightly 3-lobed and clothed with small orbicular scales. In each fruit, the numbers of seeds are eight that are 6.0 mm long, rounded and smooth on the back.

The pictures of stem bark, leaf, flower and fruit of *Croton oblongifolius* Roxb. are shown in Fig.1[10].

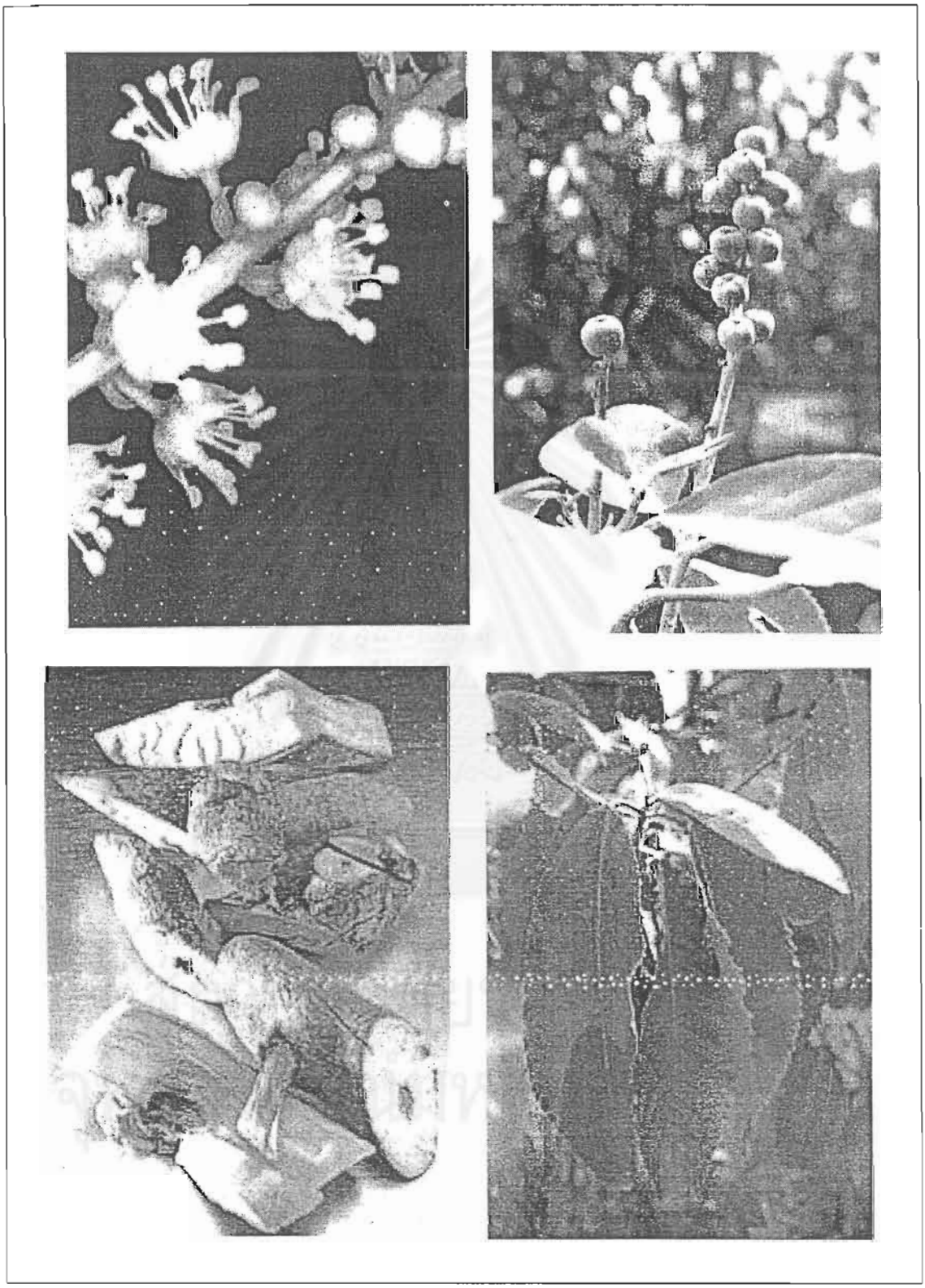


Figure 1 The stem bark, leaves, flowers and fruits of *Croton oblongifolius*

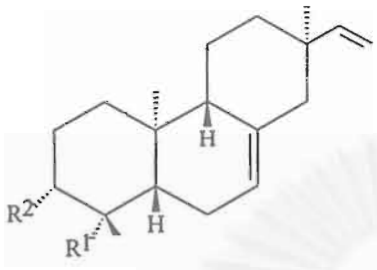
From previous studies, chemical constituents of *Croton oblongifolius* Roxb. in Thailand are differently. In order to find reliable sources of chemical constituents of C. oblongifolius from various locations in Thailand were investigated by NMR screening of the crude hexane extract. It turns out that spectrum of crude hexane extract from amphur Wang Sa Phung, Loei province were difference from spectral data of others. Therefore, it is of more interested in to study chemical components as well as their biological activities in C. oblongifolius from amphur Wang Sa Phung, Loei Province

Thus, the objective of this research will be summarized as follows

1. To extract and isolate the chemical constituents of the stem bark of *Croton oblongifolius* Roxb.

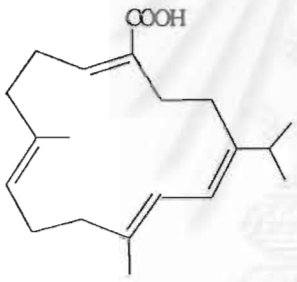
2. To identify the structure formula of the isolated substances.

Isopimarane group

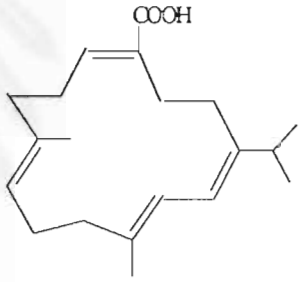


R^1	R^2	
CH_2OH	OH	Oblongifoliol
CH_3	OH	19-Deoxyoblongifoliol
COOH	H	Oblongifolic acid
CH_3	H	ent-Isoprimara-7-15-diene
CHO	H	ent-Isoprimara-7-15-diene-19-aldehyde

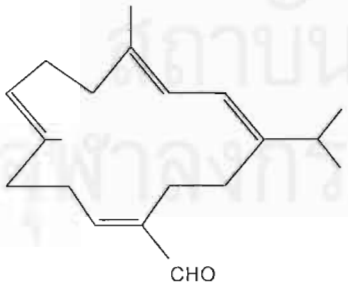
Cembrane group



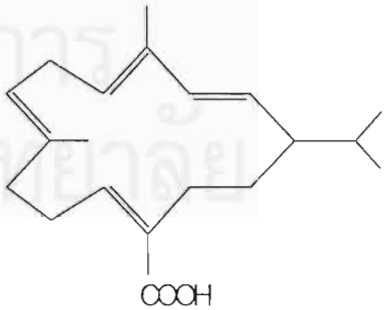
Crotocembraneic acid



neo-Crotocembraneic



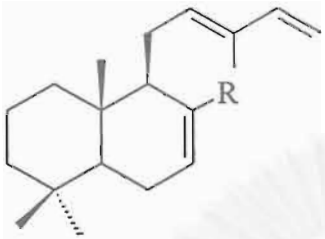
Crotocembranal



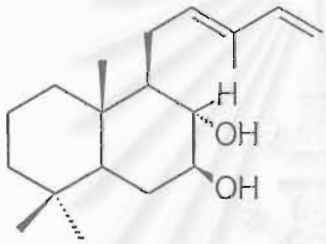
Poilaneic acid

Figure 2 The structure of the chemical constituents of *Croton oblongifolius* Roxb.

Labdane group

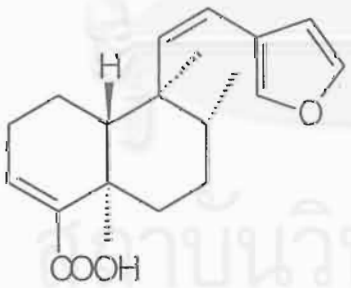


R	
CH_3	labda-7,12(*E*),14-triene
CHO	labda-7,12(*E*),14-triene-al
CH_2OH	labda-7,12(*E*),14-triene-ol
COOH	labda-7,12(*E*),14-triene-oic acid

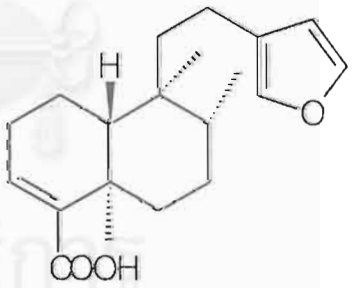


Nidorellol

Clerodane group



11-Dehydro(-)-hardwickiic acid



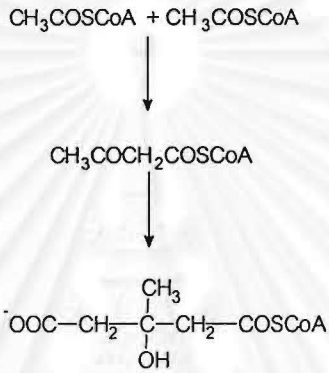
(-)-Hardwickiic acid

Figure 2 The structure of the chemical constituents of *Croton oblongifolius* Roxb.

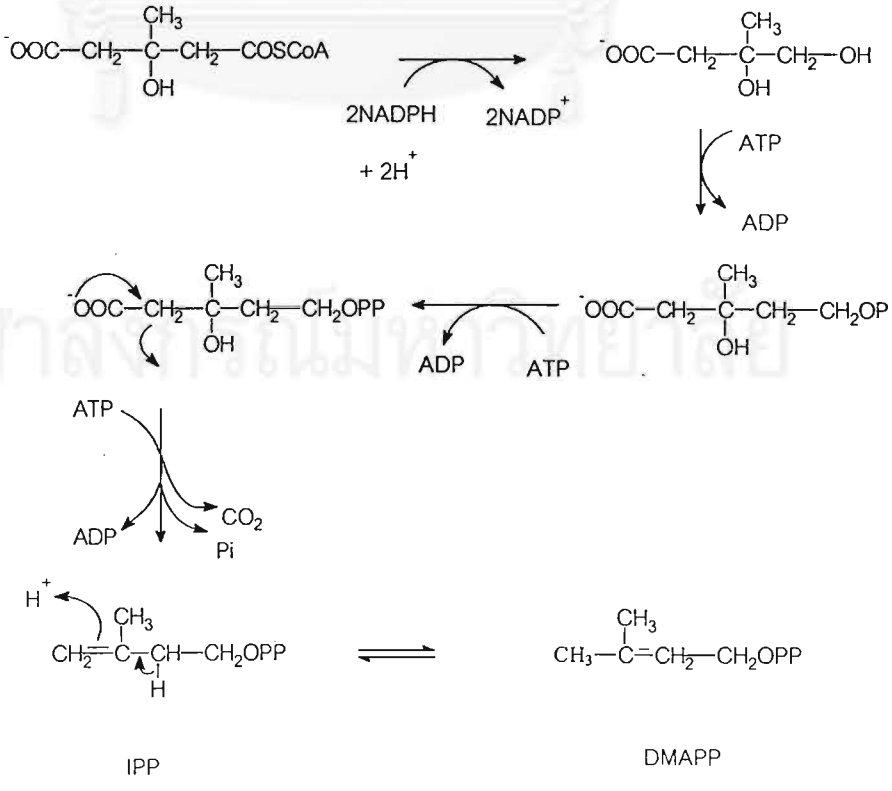
Biological synthesis of diterpenoid compounds

Biological synthesis of diterpenoid compound was shown in Scheme 1-3 [17]

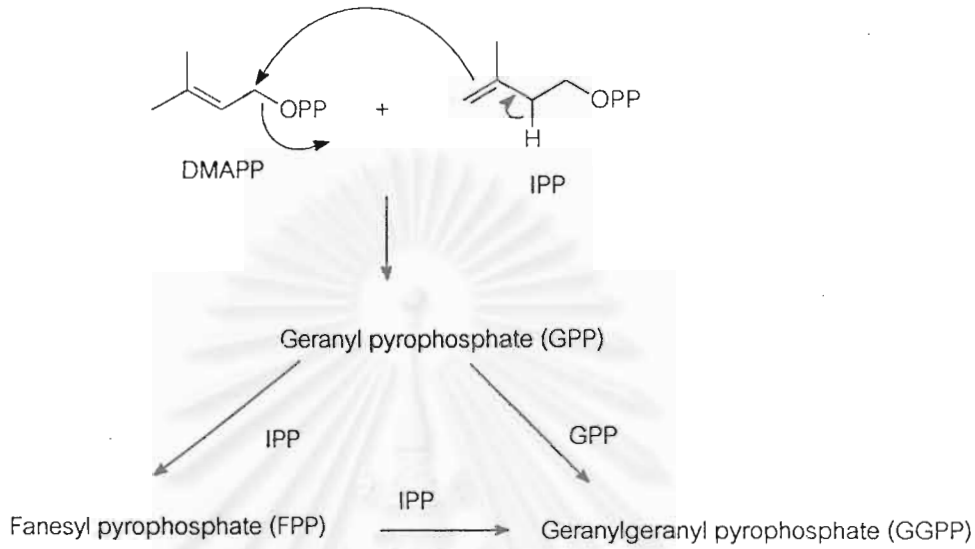
Scheme 1. Biosynthesis of (s)-3-hydroxy-3methylglutaroyl coenzyme A.



Scheme 2. Origin of The isopentenyl-pyrophosphate (IPP) and dimethyl allylpyrophosphate (DMAPP) units.



Scheme 3. Assembly of isopenes.



From scheme 3, geranylgeranyl pyrophosphate can cyclization [18] gives many diterpenoid compounds such as labdane, clerodane, cembrane etc. In *C. oblongifolius* Roxb. can found several diterpenoid compounds.

Biological activity of diterpenoid compound isolated from *C. oblongifolius*

Diterpenoid compounds from C. oblongifoius Roxb. have biological activity such as cytotoxicity, cAMP phosphodiesterase inhibition, antimicrobial antiplatelet aggregation etc. For labdane, the compounds from Prachuab Kirikhan [15] have active with against human tumor cell lines and have antiplatelet aggregation. For clerodane, hardwickckic acid [19] has antimicrobial. For cembrane, neocrotocembranal [14] have active with against human tumor cell lines, crotocembraneic acid, neocrotocembraneic acid [14] and poilaneic acid have cAMP phophodiesterase inhibition.

CHAPTER III

EXPERIMENTS

Plant Materials

The stem barks of *Croton oblongfolius* Roxb.(plao-yai) were collected from amphur Wang Sa Phung, Loei province, Thailand, in May 1998. Botanical identication was achieved through comparison with a voucher specimen No. BKF 084729 in the herbilium collection of the Royal Forest Department of Thailand.

Instruments and Equipments

1. Foufier Transform Infrared Spectrophotometer (FT-IR)

The FT-IR spectra were recorded on a Nicolet Impact 410 Spectrophotometer. Spectra of solid samples were recorded as KBr pellets and liquid samples were recorded as thinfilm on KBr cells.

2. Mass Spectrometry (MS)

The mass spectra were recorded on a Fisons Instruments Mass Spectrometer Model Trio 2000 in EI mode at 70 eV

3. Ultraviolet-Visible Spectrometry (UV-VIS)

The UV-VIS spectra were recorded on a Hewlett Packard 8452 A diode array spectrophotometer in chloroform.

4. ^{1}H and ^{13}C Nuclear Magnatic Resonance Spectrometer (NMR)

The ^{1}H and ^{13}C Nuclear Magnatic Resonance Spectra were recorded at 200.13 and 50.32 MHz, respectively, on a Bruker Model AC-F200 Spectrometer and at 500.00 and 125.65 MHz on a JEOL JNM-A500 spectrometer in $CDCl_3$. Chemical shifts are given in parts per million using residual protonated solvent as reference. COSY, NOESY, HMQC and HMBC experiments were performed on the JEOL JNM-A500 spectrometer.

5. Elemental Analysis (EA)

The EAs were measured on a Perkin Elmer PE2400 SERIES II (CHN/O ANALYSER).

6. Optical rotation

The optical rotation were measured on a Perkin-Elmer 341 polarimeter in $CHCl_3$.

Chemical Reagents

1. Solvents

All solvents used in this research such as hexane, chloroform, ethyl acetate and methanol were commercial grade and were purified prior to use by distillation.

2. Other chemicals

2.1 Merck's silica gel 60 G Art. 7734 (70-230 mesh ASTM) and 9385 (230-400 mesh ASTM) were used as adsorbents for normal column chromatography and flash column chromatography.

2.2 Merck's TLC aluminum sheet, silica gel 60 F_{254} precoated 25 sheets, 20x20 cm^2, layer 0.2 mm was used to identify the identical fractions

Extraction and Isolation

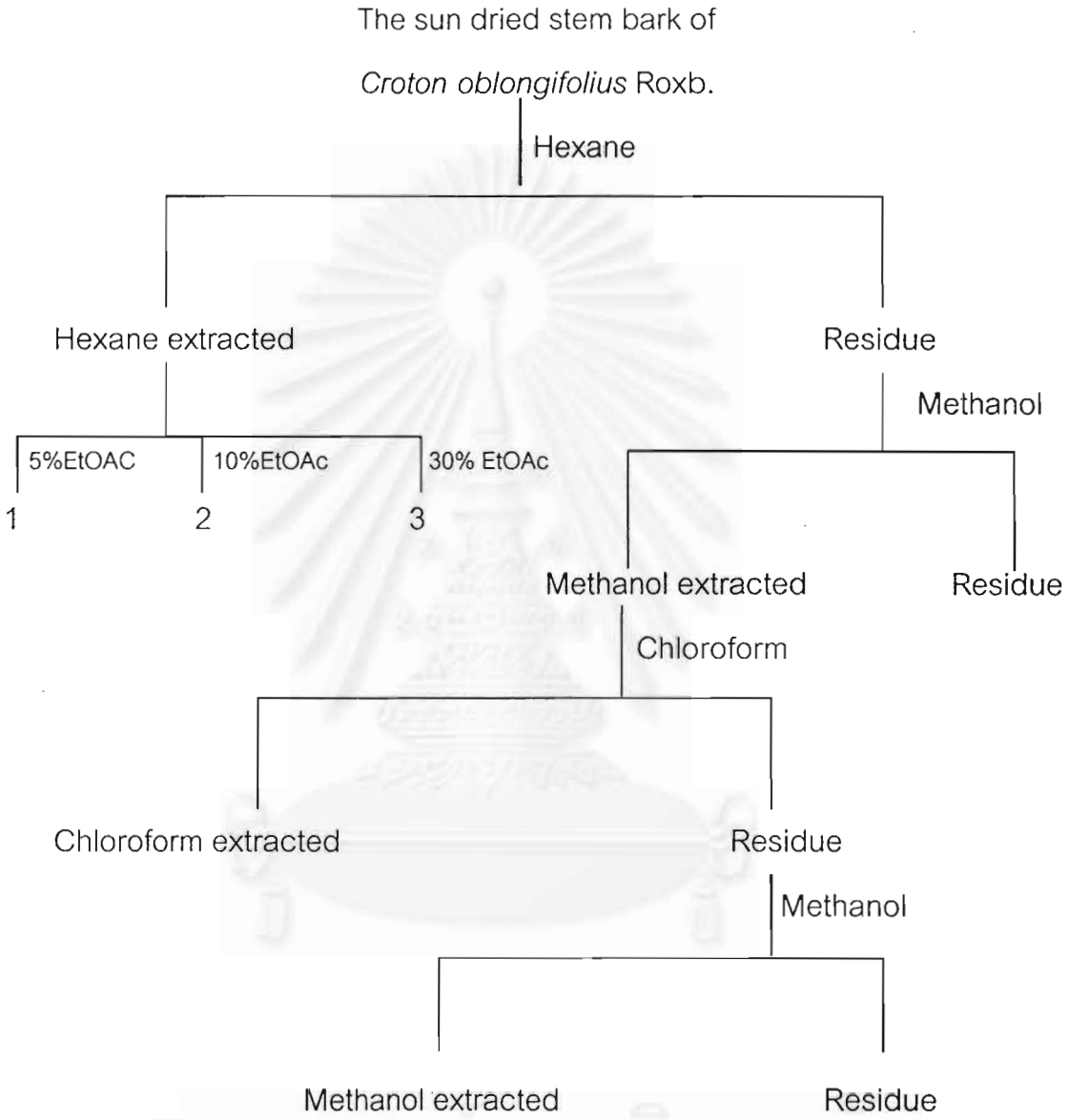
The powdered, sun dried stem barks (6.5 kg.) of *Croton oblongifolius* Roxb. was extracted with hexane. The hexane solution was filtered and evaporated under reduced pressure to obtain yellowish green oil (300 g). The residue, after hexane extraction, was extracted with methanol. The methanol solution was filtered and evaporated under reduced pressure to obtain dark-red gummy residue (300 g) which was repeatedly re-extracted with chloroform and methanol respectively. The chloroform and methanol solution was filtered and evaporated under reduced pressure to obtain dark brown oil and dark-red oil, respectively.

Three different crude extracts of the stem barks of *C. oblongiolius* are as shown in Table 2 and the extraction procedure are shown in Scheme1.

Table 2 The various extracted of the stem bark of *C. oblongifolius* Roxb.

Solvent extract	Appearance	Weight(g)	%wt/wt of the dried stem bark
Hexane	yellowish green oil	300	4.61
Chloroform	dark brown oil	80	1.23
Methanol	dark red oil	120	1.85

Scheme 4 The extraction procedure of the stem bark of *C.oblongifolius* Roxb.



Isolation of the chemical constituents from the stem barks of *Croton oblongifolius* Roxb.

Separation of hexane crude extract

The hexane crude extract (100g) was separated by column chromatography. The column was packed with silica gel 60 Art.7734 (70-230 mesh ASTM) and the crude extract, which was adsorbed on silica gel, was added to the top of column. The column was eluted with hexane, hexane-chloroform gradient in a stepwise fashion. The volume of eluting solvent was approximately 500 cm^3 and its was evaporated to about 30 cm^3. Fractions with similar components were combined.

Separation of chloroform crude extract

The chloroform crude extract (80g) was separated by column chromatography on silica gel 70-230 mesh ASTM. The column was eluted with hexane, hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol respective. The 200 cm^3 eluted fraction was collected and it was concentrated to about 30 cm^3, then analyzed by TLC. Fractions with similar components were combined.

Separation of methanol crude extract

The methanol crude extract was gummy residue that insoluble in all solvent. Therefore, it was not separate by column chromatrography.

Purification and properties of the compounds eluted from column chromatography of hexane crude extract.

Purification and properties of Compound 1

Compound 1 was eluted with 10% chloroform in hexane. The solvent was removed by rotatory evaporation and the residue was purified by column chromatography (Merck's silica gel Art.1.09385.1000) eluting with 5% ethyl acetate in hexane. It is soluble in dichloromethane, chloroform, ethyl acetate, diethyl ether and methanol.

Compound 1 is a white solid (120 mg, 0.005%), $[\alpha]_D^{20}$ +50.17 ($CHCl_3$;c 1.0), mp. 102-103°C, UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244sh (4.45), EA; found C 76.24%; H 9.91% calc: C 76.26%; H, 9.89%.

FTIR spectrum(KBr) (Fig 16, Table 3) ν_{max} (cm^{-1}): 3463 (OH), 2974, 2940, 2863, 1722 (C=O), 1639 (C=C), 1441, 1373, 125 2 (C-O), 1030.

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz)(Fig.18, Table4, 5) δ(ppm): 6.31 (1H,dd), 5.35(1H,t), 5.05(1H,d), 4.96(1H,ddd), 4.89(1H,dd), 4.87(1H,br.d), 4.49 (1H,br.d), 3.23(1H,dd), 2.42(1H,ddd), 2.29(1H,br.dd), 2.19(1H,dd), 2.12(1H,dd), 2.10(3H,s), 2.00(1H,dd), 1.79(1H,br.d), 1.74(3H,d), 1.74(1H,m), 1.41(1H,dddd), 1.25(1H,dd), 1.22(1H,dd), 1.06(3H,s), 0.87(3H,s), 0.85(3H,s).

^{13}C-NMR spectrum ($CDCl_3$, 125.65 MHz) (Fig.19, Table 5) δ(ppm): 171.6 (q), 146.3(q), 141.4(d), 133.7(q), 133.1(d), 110.1(t), 108.8(t), 80.5(d), 73.2(d), 56.6(d), 54.4(d), 42.3(t), 40.2(q), 39.9(q), 37.6(t), 28.7(q), 23.5(t), 23.4(t), 21.4 (q), 16.5(q), 15.2(q), 11.9(q).

EIMS (Fig. 17) *m/z* (rel. int.): 346[M^+] (24), 331[M^+-CH_3](6), 328[M^+-H_2O] (4), 317(7), 304(8), 290(15), 286[M^+-CH_3COOH](75), 272(16), 271(72), 268(63),

255(25), 253(100), 243(49), 230(28), 229(43), 225(34), 213(33), 191(30), 187 (46), 185(32), 173(37), 171(27), 147(31), 145(41), 133(32), 131(40), 119(40), 105(57), 91(70), 79(42), 77(66).

Purification and properties of Compound 2

Compound 2 was eluted with 10% chloroform in hexane. Similar fractions were combined and the solvents were removed by rotatory evaporation and further purified by column chromatography (Merck's silica gel Art.1.09385.1000) eluting with 10% ethyl acetate in hexane. This compound is soluble in dichloromethane, chloroform, ethyl acetate, diethyl ether and methanol.

Compound 2 is a white solid (60mg, 0.003%), $[\alpha]_D^{20}$ +9.46° ($CHCl_3$; c 1.0), mp. 99-101°, UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244sh (4.53), EA; found C 76.25% H 9.89% calc C 76.26% H, 9.89%.

FTIR spectrum (KBr)(Fig. 25, Table 6) ν_{max}^{KBr} cm^{-1}: 3443 (OH), 2940, 2848, 1722 (C=O), 1644, 1605, 1460, 1373, 1248 (C-O), 1030.

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz)(Fig. 27, table 7, 8) δ(ppm): 6.32 (H,dd), 5.40 (H,t), 5.05 (1H,d), 4.89 (1H,ddd), 4.87 (1H,br.d), 4.55 (1H,d), 4.51 (1H,br.d), 3.81 (1H,ddd), 2.41 (1H,ddd), 2.36 (1H,m), 2.21 (1H,dd), 2.19 (1H,m), 2.15 (3H,s), 2.02 (1H,m), 1.80 (1H,br.d), 1.75 (3H,d), 1.72(1H,dddd), 1.41 (1H,dddd), 1.29 (1H,dd), 1.29 (1H,dd), 0.90 (3H,s), 0.87 (3H,s) 0.80 (3H,s).

^{13}C-NMR spectrum ($CDCl_3$, 125.65 MHz) (Fig.28, Table 8) δ(ppm): 172.4 (q), 146.9 (q), 141.5 (d), 133.8 (q), 133.0 (d), 110.2 (t), 108.8 (t), 84.5 (d), 67.8 (d), 56.6 (d), 54.4 (d), 46.3 (t), 40.1 (q), 39.4 (q), 37.6 (t), 28.7 (q), 23.5 (t), 23.3 (t), 21.2 (q), 17.5 (q), 15.4 (q), 11.9 (q).

EIMS (Fig. 26) *m/z* (rel. int.):346[M^+] (9), 328[M^+-H_2O] (4), 313 (1), 286 [M^+-CH_3COOH] (19), 271 (45), 268 (32), 255 (22), 253 (78), 243 (39), 229 (44), 213 (32), 203 (27), 187 (52), 173 (67), 159 (56), 147 (68), 145 (60), 135 (73), 133 (87), 121 (80), 119 (95), 107 (82), 105 (100), 95 (53), 93 (68), 91 (70), 81 (67), 79 (89).

Purification and properties of Compound 3

Compound 3 was eluted with 30% chloroform in hexane. The solvent was removed by rotatory evaporation and the residue was purified by column chromatography (Merck's silica gel Art.1.09385.1000) eluting with 30% ethyl acetate in hexane. It is soluble in dichloromethane, chloroform, ethyl acetate, diethyl ether and methanol.

Compound 3 is a white solid (8.49 g, 0.413%), $[\alpha]_D^{20}$ +-6.96 ($CHCl_3$;c 1.0), mp. 69-70°c, UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 245.5sh (4.14), EA; found C 78.87%; H 10.59% calc: C 78.90%; H, 10.59%.

FT-IR spectrum (KBr) (Fig.34, Table9) ν_{max} (cm^{-1}): 3376 (OH), 2969, 2945, 2853, 1644, 1610, 1446, 1388, 1214 (C-O), 1059.

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz)(Fig.36, Table 10, 11) δ(ppm): 6.29 (1H,dd), 5.38 (1H,t), 5.02 (1H,d), 4.86 (1H,d), 4.85 (1H,br.d), 4.47 (1H,br.d), 3.69 (1H,ddd), 3.02 (1H,d), 2.39 (1H,ddd), 2.34 (1H,br.dd), 2.17 (1H,dd), 2.10 (1H,dd), 1.99 (1H,m), 1.76 (1H,br.d), 1.72 (3H,d), 1.71 (1H,m), 1.40 (1H,dddd), 1.19 (1H,dd), 1.18 (1H,dd), 1.01 (3H,s), 0.80 (3H,s), 0.78 (3H,s).

^{13}C-NMR spectrum ($CDCl_3$, 125.65 MHz) (Fig.37, Table 11) δ(ppm): 147.2 (q), 141.5 (d), 133.7 (q), 133.2 (d), 110.1 (t), 108.6 (t), 83.5 (d), 69.1 (d),

56.7 (d), 54.5 (d), 45.0 (t), 40.1 (q), 39.3 (q), 37.7 (t), 28.8 (q), 23.6 (t), 23.3 (t), 16.6 (q), 15.4 (q), 11.9 (q).

EIMS (Fig 35) *m/z* (rel. int.): 304[M^+] (68), 289[M^+-CH_3] (53), 286[M^+-CH_3COOH] (22), 271 (75), 253(33), 248 (62), 233 (45), 215 (27), 201 (26), 187 (78), 173 (42), 159 (46), 147 (61), 145 (70), 133 (76), 119 (76), 105 (94), 91 (100), 79 (84).

Acetylation of compound 3:

The compound 3 was acetylation with acetic anhydride in pyridine, stir at room temperature.

A: with 1.2 equivalent of acetic anhydride: Acetic anhydride (40 mg, 0.39 mmol) was added slowly into a stirred solution of compound 3 (100 mg, 0.33 mmol) in 4 ml of dry pyridine. After the solution was completed, the reaction mixture was stirred for 6 hours at room temperature. The reaction was stopped and worked up by usual manner. The organic layer was concentrated by rotatory evaporation and purified by column chromatography (Merck's silica gel Art.1.09385.1000) eluting with 5% ethyl acetate in hexane to yield compound 1 (45 mg, 39.8 %) and compound 2 (35 mg, 31.5%). The spectral data (^1H and ^{13}C NMR, IR and MS) of these compounds were identical to that of naturally occurring.

B: with 2.4 equivalent of acetic anhydride: Acetic anhydride (80 mg, 0.78 mmol) was added slowly into a stirred solution of compound 3 (100 mg, 0.33 mmol) in 5 ml of pyridine. After the solution was completed, the reaction mixture was stirred for 6 hours at room temperature. Purification by column chromatography (Merck's silica gel Art.1.09385.1000) eluting with 2% ethyl

acetate in hexane to yield compound 4 (85 mg, 66.0 %). Compound 4 was white solid $[\alpha]_D^{30}$ =+24.52 (c=1.0) Rf = 0.8 (20% ethyl acetate in hexane), UV λ_{max} (EtOH) 232sh (logε 4.39)

FTIR spectrum (KBr)(Fig. 43, Table 12) $\nu_{max}(cm^{-1})$: 2979, 2955, 2850, 1732(C=O), 1645, 1613, 1440, 1384, 1255(C-O),1040.

^{1}H NMR spectral ($CDCl_3$, 500 MHz) (Fig. 45, Table 13,14) δ (ppm) : 6.25 (1H,dd), 5.28(1H,dd), 5.04(1H,ddd), 4.98(1H,d), 4.81(1H,d), 4.80(1H,m), 4.71 (1H,d), 4.43(1H,br.d), 2.34(1H,ddd), 2.20(1H,m), 2.12(1H,m), 2.08(1H,dd), 1.99 (3H,s), 1.96(1H,m), 1.93(3H,s), 1.73(1H,br.d), 1.66(3H,d), 1.65(1H,m), 1.35 (1H,dddd), 1.27(1H,dd), 1.25(1H,dd), 0.84(3H,s), 0.83(3H,s), 0.80(3H,s).

^{13}C NMR spectral ($CDCl_3$, 125.65 MHz) (Fig. 46, Table 14) δ (ppm) : 170.5(q), 170.3(q),146.6(q), 141.3(d), 133.8(q), 132.8(d), 110.1(t), 108.9(t), 80.2 (d), 70.0(d), 56.5(d), 54.1(d), 42.4(t), 39.9(q), 39.3(q), 37.4(t), 28.5(q), 23.4(t), 23.3(t), 21.0(q), 20.8(q), 17.5(q), 15.2(q), 11.9(q).

EIMS (Fig 44) m/z (rel. int.) : 388[M^+] (11), 373(1), 328[M^+-CH_3COOH] (25), 313 (13), 286 (17), 268[M^+-2CH_3COOH] (70), 253 (100), 239 (15), 225 (33), 211 (38), 197 (38), 187 (52), 173 (33), 159 (23), 145 (39), 133 (28), 119 (51), 105 (62), 91 (60), 79 (63).

Purification and properties of the compounds eluted from column chromatography of chloroform crude extract.

The spectral data [^{1}H and ^{13}C NMR spectra] of chloroform crude extract were similar to hexane crude extract. Purification by column chromatography give compound 3 (0.98 g, 0.015 %)

Biological evaluation

Samples were also tested for cytotoxic activity towards 6 cell lines, which contain L929 (fibroblast), S102 (hepatoma), HEP-G2 (heptatoma), SW 620 (colon), chago (lung), Kato-3 (gastric) and BT 474 (breast) following the experimental method of bioassay of cytotoxic activity against 6 cell cultures *in vitro* were performed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method.[20,21]

CHAPTER IV

RESULT AND DISCUSSION

Crude Hexane Extract

The crude hexane extract of the stem bark of *C. oblongifolius* Roxb. was separated by chromatography on a silica gel column using a hexane-ethyl acetate gradient system to obtain three new labdane diterpenoids **1-3**. Treatment of **3** with acetic anhydride in pyridine gave **4**.

1. Structure Elucidation of compound 1

The molecular formula of compound **1** was assigned as $C_{22}H_{34}O_3$ based on elemental analysis, IR, ^1H and ^{13}C NMR and EIMS.

The EIMS spectrum of compound **1** exhibited at $[M^+]$ *m/z* 346. The ion at *m/z* 331, 328 and 286 were formed by loss of CH_3 group, H_2O group and acetic acid, respectively.

The IR spectrum of compound **1** was summarized in Table 3.

Table 3. The IR absorption band assignment of compound **1**.

Wave Number (cm^{-1})	Intensity	Tentative assignment
3463	Medium	OH stretching vibration of alcohol
2974,2940, 2863	Strong	CH stretching vibration of $-CH_2$, $-CH_3$
1722	Strong	C=O stretching vibration
1639	Medium	C=C stretching vibration
1441, 1373	Medium	$-CH_2$, $-CH_3$ bending
1253	Medium	C-O stretching vibration

The information from 2D-NMR techniques, COSY correlations (Fig. 23, Table 4), HMQC correlations (Fig. 21, Table 4), HMBC correlations (Fig. 22, Table 4) were used to assist the structure assignment of compound 1.

The ^{1}H-NMR spectrum (Fig. 18, Table 4) of compound 1 showed four methyl groups attaching to quaternary carbons (0.85, 0.87, 1.06 and 2.10 ppm) and one olefinic methyl groups (1.74 ppm) and six olefinic protons (6.31, 5.35, 5.06, 4.89, 4.88 and 4.49 ppm) and a double doublet at 6.31 ppm.

The ^{13}C-NMR spectrum (Fig. 19) and DEPT experiments revealed the presence of 22 nonequivalent carbons. The DEPT 90 (Fig. 20), indicated the presence of two sp^2 methine carbon at 141.4 and 133.2 ppm and four saturated methine carbons at 80.5, 73.2, 56.6 and 54.4 ppm. The DEPT 135 spectrum (Fig 24) showed six methylene carbons at 110.1, 108.8, 42.3, 37.6, 23.5 and 23.4 ppm and five methyl carbons at 28.7, 21.4, 16.5, 15.2 and 11.9 ppm (table 4,5). From ^{13}C-NMR, indicated that the carbon signals at 171.6, 146.3, 133.7, 40.2 and 39.9 ppm were quaternary carbons. The six vinylic (or sp^2) carbons were consistent with the presence of three double bonds in the molecule. The molecular formula, $C_{22}H_{34}O_3$ of compound 1 defined a degree of unsaturation of six, therefore compound 1 must consist of two rings in addition to the three double bonds and one carbonyl group.

The ^{1}H and ^{13}C-NMR suggested that compound 1 possesses an acetate group (δ_H 2.10 and δ_C 21.4 ppm for $\underline{C}H_3CO$), consistent with the EIMS data. The -CH=C(CH_3)-CH=CH_2 group gave a typical ^{1}H-NMRspectrum for a labda-12(*E*),14-diene skelaton (δ_H 4.89, H-15, d, *J*=11.0; 5.05, H-15, d, *J*=17.4; 5.35, H-12, dd, *J*=6.4 and 6.4; 6.31, H-14, dd, *J*=11.0 and 17.4; and 1.74, H_3-16, d, *J*=0.9)[12,22,23]. The exomethylene group showed ^{1}H and ^{13}C-NMR signals at δ_H 4.49 (H-17, br.d, *J*=1.5), 4.87 (H-17, br.d, *J*=1.5), and δ_C

108.8 (C-17,t). The ^{1}H-NMR spectrum also showed three additional singlets due to three methyl groups at δ_H 0.85 (H_3-20), 0.87 (H_3-19) and 1.06 (H_3-18). Therefore, the OH and acetoxy moieties must be confined to the decalin ring system. A well-defined double doublets at δ_H 3.23 (H-3, *J*=5.2,10.0) was shown to be on C-3 carbon (δ_C 80.5) by HMQC experiment and it was shown to be correlated to a quaternary carbon at δ_C 39.9 (C-4), two methyl carbons (δ_C 16.5, C-19 and 28.7, C-18) and an oxygen bearing methine carbons at δ_C 73.2 (C-2) by HMBC experiment. Thus the two oxygen bearing methine carbons must be adjacent to one another. The well-defined doublet of double doublets at δ_H 4.96 (H-2, J=4.3, 10.0 and 11.7) was shown to connect to δ_C 73.2 (C-2) by HMQC and correlated to δ_C 42.3 (C-1), 80.5 (C-3) and 171.6 (C=O) by HMBC experiment. Therefore, the acetoxy group must be on C-2 carbon. A large coupling constant of 10.0 Hz [14] between H-2 and H-3 indicated that both of them were axial orientation. Thus, the OH and acetoxy moieties were in equatorial orientation. The unequivocal assignment of compound 1 was established by the information from HMQC, HMBC, COSY and NOESY experiments as in Figure 3-6 and Table 4.

The spectral data of 2,3 dioxygenated decalin ring system with 8(17) exomethylene of compound 1 was also in agreement with those of methyl 2β,3β-dihydroxy-labda-8(17),13*Z*-diene-15-oate isolated from *Nolana rostrata*[25]. Thus, the structure of compound 1 was proposed to be 2-acetoxy-labda-8(17),12(*E*),14-triene-3-ol as shown in Figure 3. The long-range C-H correlations, COSY correlations, and NOESY correlations by HMBC spectrum were sumerize in Figure 4-6.

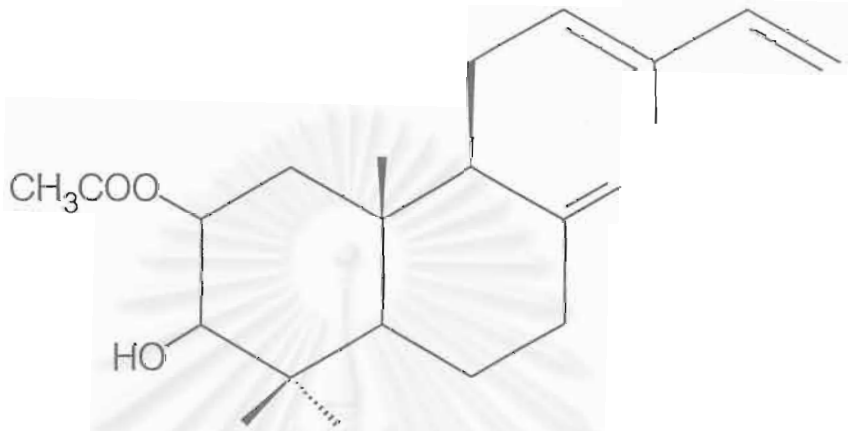


Figure 3 The structure of compound 1

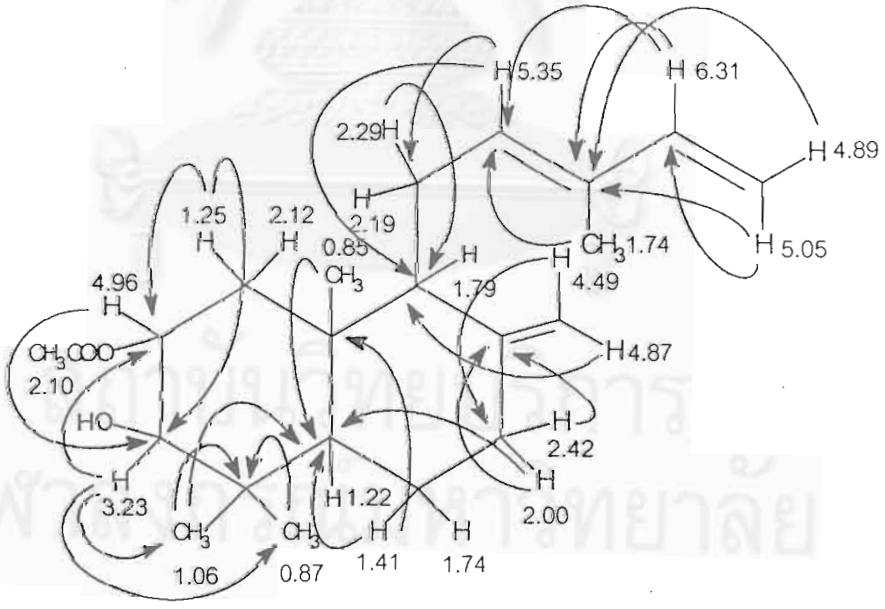


Figure 4 The HMBC correlations of compound 1

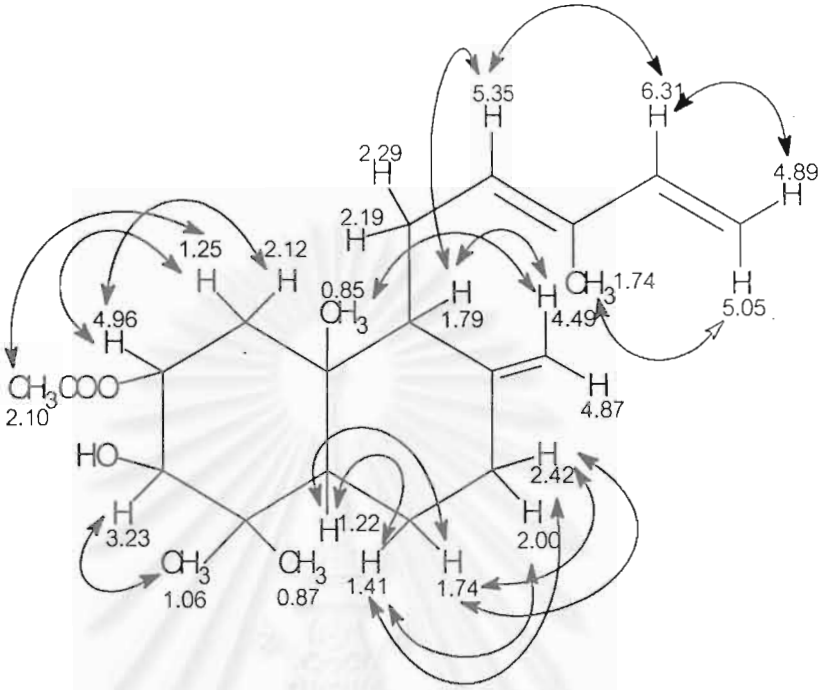


Figure 5 The COSY correlations of compound 1

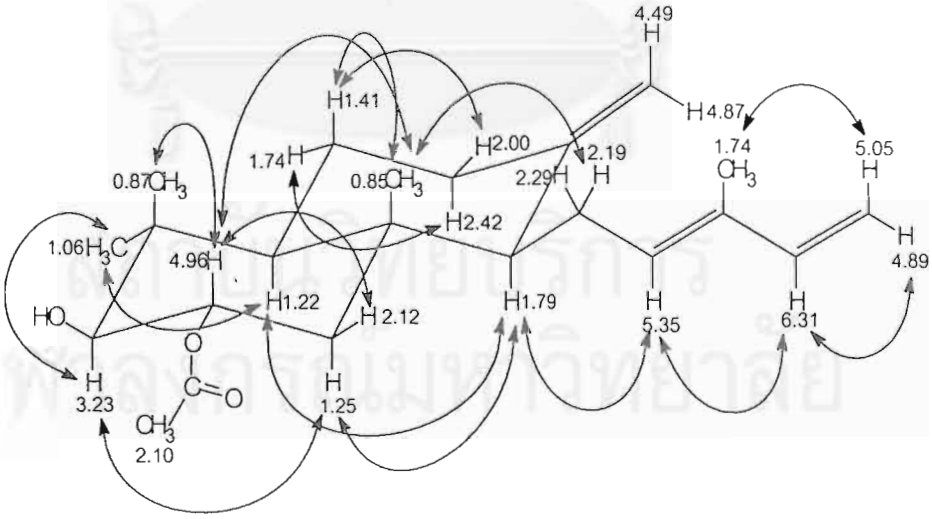


Figure 6 The NOESY correlations of compound 1

Table 4. The HMQC, HMBC, and COSYspectral data of compound 1

Position	δ_C [a]	δ_H	HMBC (H to C)	COSY
1	42.3(t)	1.25 dd (*J*=11.7, 11.7)	C-2,C-3,C-9,C-10,C-20	4.96, 0.85
		2.12 dd (*J*=4.3,11.7)	C-2,C-3,C-5,C-10,C-20	2.10
2	73.2(d)	4.96 ddd (*J*=4.3,10.0,11.7)	C-1,C-3,C-18,C=O	0.85, 2.12
3	80.5(d)	3.23 dd (*J*=5.23, 10.0)	C-2,C-4,C-18,C-19	1.06
4	39.9(s)	-	-	-
5	54.4(d)	1.22 dd (*J*=2.7, 12.6)	C-4,C-6,C-9,C-10,C-18,C-19,C-20	1.41, 1.74
6	23.5(t)	1.41 dddd (*J*=4.3, 12.6, 12.6, 12.6)	C-5,C-7,C-8,C-10	2.00, 2.42, 1.22
		1.74 m	C-4,C-5	
7	37.6(t)	2.00 dd (*J*=5.2, 13.1)	C-6,C-8	1.41, 1.74
		2.42 ddd (*J*=2.4, 4.3, 13.1)	C-5,C-6,C-8,C-9	1.41, 1.74
8	146.3(s)	-	-	-
9	56.6(d)	1.79 br.d (*J*=11.6)	C-8,C-10,C-11,C-20	4.49, 5.35
10	40.2(s)	-	-	-
11	23.4(t)	2.19 dd (*J*= 6.4, 10.6)	C-8,C-9	4.49
		2.29 br.dd (*J*=4.3, 15.0)	C-8,C-9,C-12,C-13	
12	133.1(d)	5.35 dd (*J*=6.4, 6.4)	C-9,C-11,C-13,C-14,C-16	6.31, 1.79
13	133.7(s)	-	-	-
14	141.4(d)	6.31 dd (*J*=11.0, 17.4)	C-12,C-13,C16	5.35
15	110.1(t)	4.89 d (*J*=11.0)	C-13	6.31, 4.49
		5.05 d (*J*=17.4)	C-13,C-14	1.74
16	11.9(q)	1.74 d (*J*=1.0)	C-11,C-12,C-13,C-14,C-15	
17	108.8(t)	4.49 br.d (*J*=1.5)	C-7,C-9	0.85, 2.19, 1.79
		4.87 br.d (*J*=1.5)	C-7,C-9	
18	28.7(q)	1.06 s	C-3,C-4,C-5,C-19	3.23
19	16.5(q)	0.87 s	C-3,C-4,C-5,C-18	
20	15.2(q)	0.85 s	C-1,C-5,C-9,C-10	4.49
C=O	171.6(s)	-	-	
$\underline{C}H_3CO$	21.4(q)	2.10 s	C=O	4.96

[a]Carbon type as determined by DEPT experiments spectra : s=singlet, d=doublet, t=triplet, q=quartet.

2. Structure Elucidation of compound 2

The molecular formula of compound 2 was assigned as $C_{22}H_{34}O_3$ based on elemental analysis, ^1H and ^{13}C-NMR, and EIMS

The EIMS spectrum of compound 2 exhibited $[M^+]$ at *m/z* 346. The ion at *m/z* 328 and 286 were formed by loss of H_2O group and acetic acid, respectively.

The IR spectrum of compound 2 was sumerized in Table 6.

Table 5. The IR absorbtion band assignment of compound 2.

Wave Number (cm^{-1})	Intensity	Tentative assignment
3443	Medium	OH stretching vibration of alcohol
2940, 2848	Strong	CH stretching vibration of $-CH_2$, $-CH_3$
1722	Strong	C=O stretching vibration
1644	Medium	C=C stretching vibration
1460, 1373	Medium	$-CH_2$, $-CH_3$ bending
1248	Medium	C-O stretching vibration

^1H and ^{13}C NMR spectra (Table 7) of compound 2 were similar to those of compound 1. The HMBC experiment revealed the correlation of methyl proton at δ_H 4.55 (H-3, d, J=10.1) to a quaternary carbon at δ_C 39.4 (C-4), an oxygen bearing methine carbon at δ_C 67.8 (C-2) and C=O carbon at δ_C 172.4. Therefore, the acetoxy group is located on the C-3 carbon. The two positions on C-2 and C-3 showed axial relationship as suggested by the large coupling constant of 10.1 Hz as in compound 1. The information of 2D-NMR including COSY correlations (Fig. 32, Table 7), NOESY correlations (Fig.33), HMQC

correlations (Fig.30, Table 8, 9) and HMBC correlations (Fig.31, Table 9) supported the structural elucidation of **2**. Thus, the structure of compound 2 was proposed to be 3-acetoxy-labda-8(17),12(*E*),14-triene-2-ol as shown in Figure 7. The long-range C-H correlations, COSY correlations, and NOESY correlations by HMBC spectrum were sumerize in Figure 9-10.

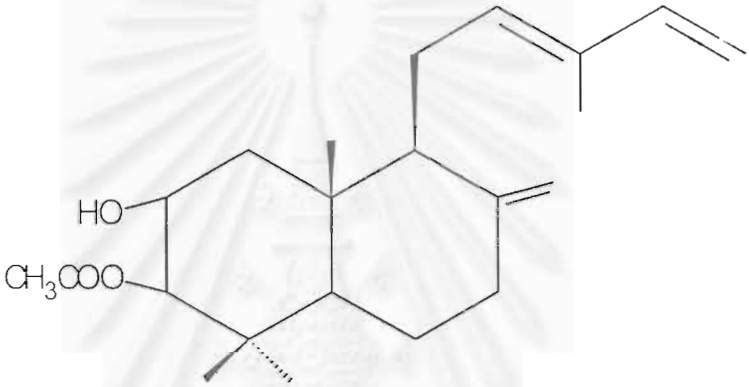


Figure 7 Structre of compound 2

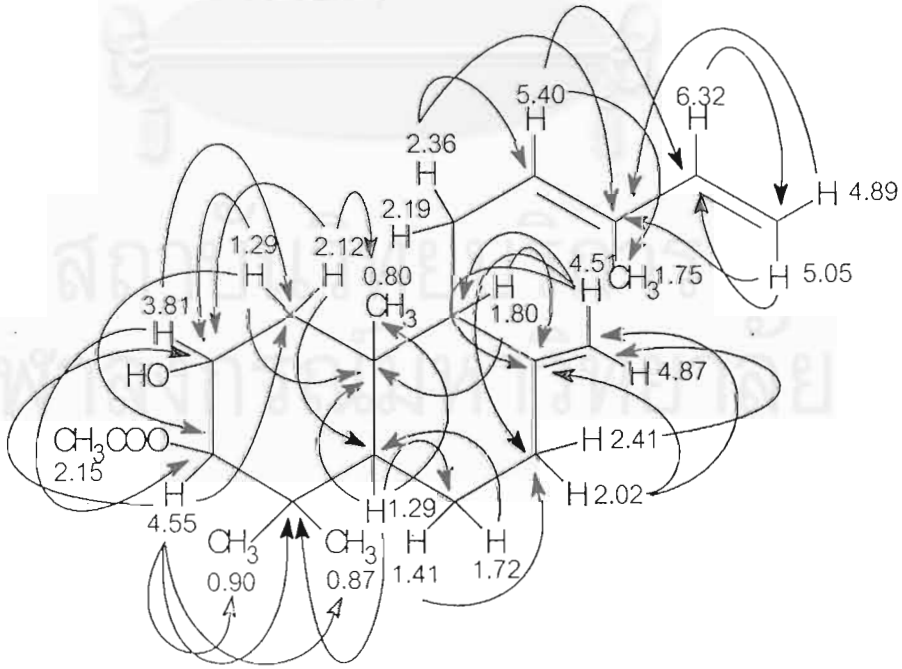


Figure 8 The HMBC correlations of compound 2

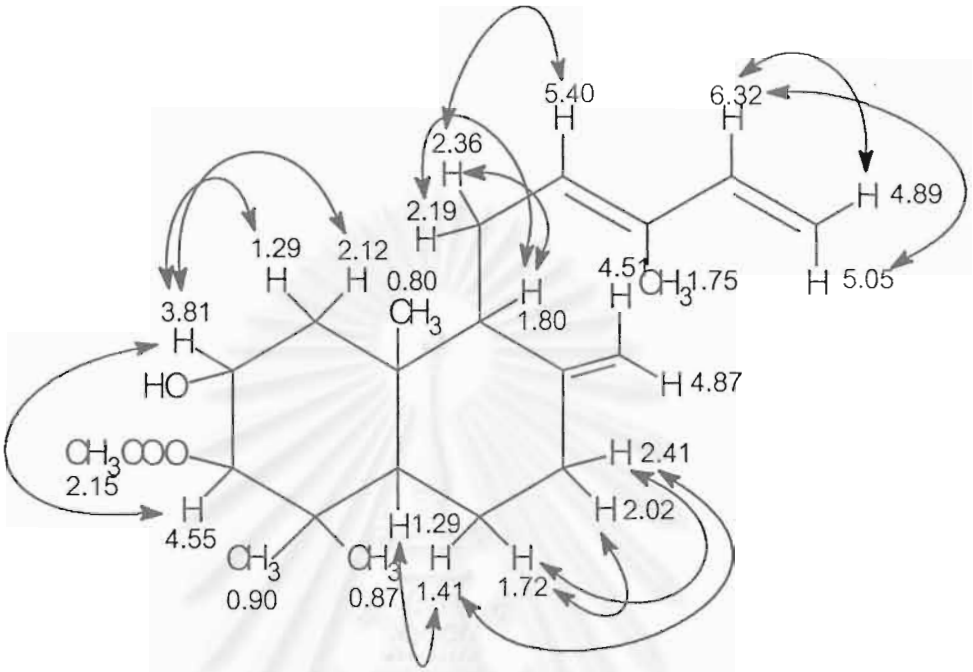


Figure 9 The COSY correlations of compound 2

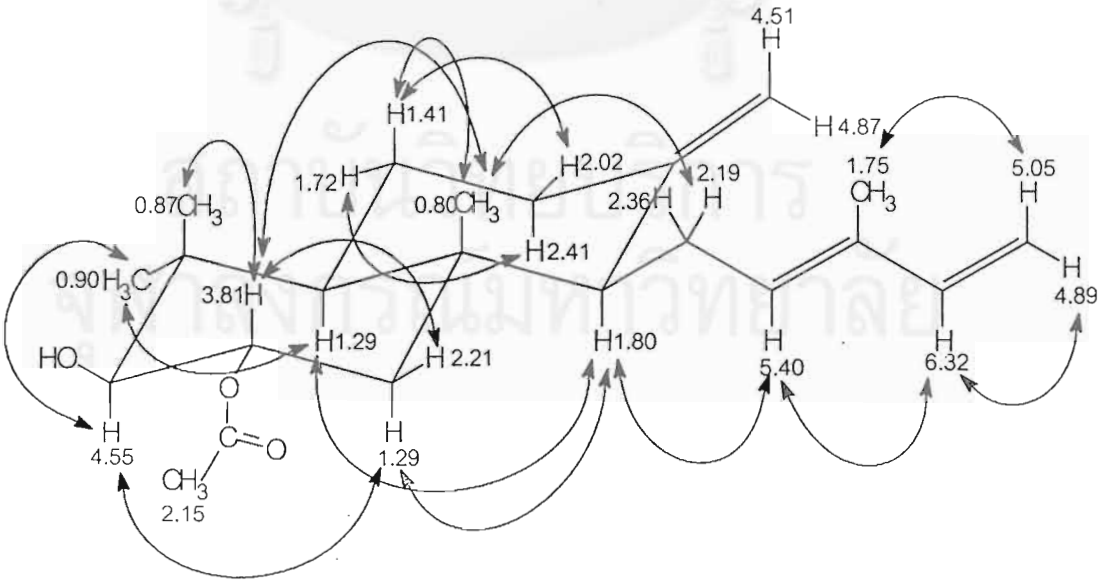


Figure 10 The NOESY correlations of compound 2

Table 6. The HMQC, HMBC, and COSYspectral data of compound 2

position	δ_C [a]	δ_H	HMBC (H to C)	COSY
1	46.3(t)	1.29 dd (*J*=12.1, 12.1)	C-2,C-3,C-9,C-10,C-20	3.81
		2.21 dd (*J*=4.3,12.1)	C-2,C-3,C-5,C-10,C-20	
2	67.8(d)	3.81 ddd (*J*=4.3,10.1,11.6)	C-1,C-3	4.45,1.27,2.21
3	84.5(d)	4.55 d (*J*=10.1)	C-2,C-4,C-18,C-19,C=O	
4	39.4(s)	-	-	-
5	54.4(d)	1.29 dd (*J*=2.4, 12.8)	C-4,C-6,C-9,C-10,C-20	1.41,1.72
6	23.5(t)	1.41 dddd (*J*=4.3, 12.8, 12.8, 12.8)	C-5,C-7	2.41,2.03
		1.74 dddd (*J*=2.6, 5.2, 5.2, 12.8)	C-5	1.29
7	37.6(t)	2.02 ddd (*J*=4.9, 13.1, 13.1)	C-6,C-8,C-17	1.41,1.72
		2.41 ddd (*J*=2.4, 4.3, 13.1)	C-5,C-8,C-9,C-17	
8	146.9(s)	-	-	-
9	56.6(d)	1.80 br.d (*J*=10.4)	C-8,C-10	2.36,2.19
10	40.1(s)	-	-	-
11	23.3(t)	2.19 m	C-8,C-9,C-12,C-13	1.80
		2.36 m	C-8,C-9,C-12,C-13	
12	133.0(d)	5.40 dd (*J*=6.4, 6.4)	C-9,C-11,C-14,C-16	2.36,2.19
13	133.8(s)	-	-	-
14	141.5(d)	6.32 dd (*J*=11.0, 17.4)	C-12,C-13,C-15,C-16	5.03,4.84
15	110.2(t)	4.89 d (*J*=11.0)	C-13	6.32
		5.05 d (*J*=17.4)	C-13,C-14	
16	11.9(q)	1.75 d (*J*=0.9)	C-12,C-13,C-14	
17	108.8(t)	4.51 br.d (*J*=1.5)	C-7,C-8,C-9	3.81,4.85
		4.87 br.d (*J*=1.5)	C-7,C-9	
18	28.7(q)	0.90 s	C-3,C-4,C-5,C-19	
19	17.5(q)	0.87 s	C-3,C-4,C-5,C-18	
20	15.4(q)	0.80 s	C-1,C-5,C-9,C-10	
C=O	172.4(s)	-	-	
$\underline{CH_3}CO$	21.2(q)	2.15 s	C=O	

[a]Carbon type as determined by DEPT experiments spectra : s=singlet, d=doublet, t=triplet, q=quartet.

3. Structure Elucidation of compound 3.

The molecular formula of compound 3 was assigned as $C_{20}H_{32}O_2$ based on elemental analysis, ^1H and ^{13}C NMR and EIMS

The EIMS spectrum of compound 3 exhibited $[M^+]$ at *m/z* 304. The ion at *m/z* 289 was formed by loss methyl group. Its mass spectrum exhibited a peak at *m/z* 286 corresponding to $[M^+-H_2O]$.

The IR spectrum of compound 3 was sumerized in Table 7.

Table 7. The IR absorbtion band assignment of compound 3.

Wave Number (cm^{-1})	Intensity	Tentative assignment
3376	strong	OH stretching vibration of alcohol
2969,2945,2853	Strong	CH stretching vibration of $-CH_2$, $-CH_3$
1644,1610	Medium	C=C stretching vibration
1446, 1388	Medium	$-CH_2$, $-CH_3$ bending
1214	Medium	C-O stretching vibration

^1H and ^{13}C NMR data for 3 were similar to those of compound 1 and 2 except the absence of acetyl group. Thus, the structure of compound 3 was proposed to be labda-8(17),12(*E*),14-triene-2,3-diol as shown in Figure 11. The long-range C-H correlations by HMBC spectrum, COSY correlations, and NOESY correlations were sumerized in Figure 12-14.

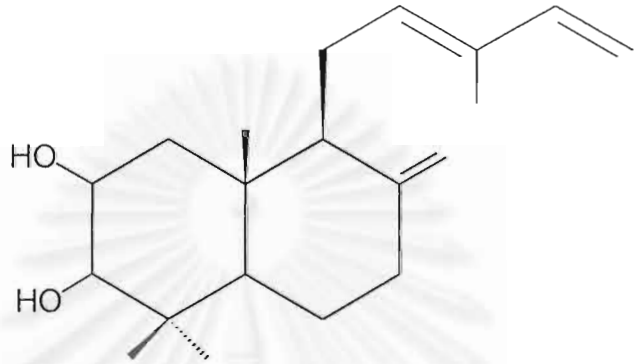


Figure 11 The structure of compound 3

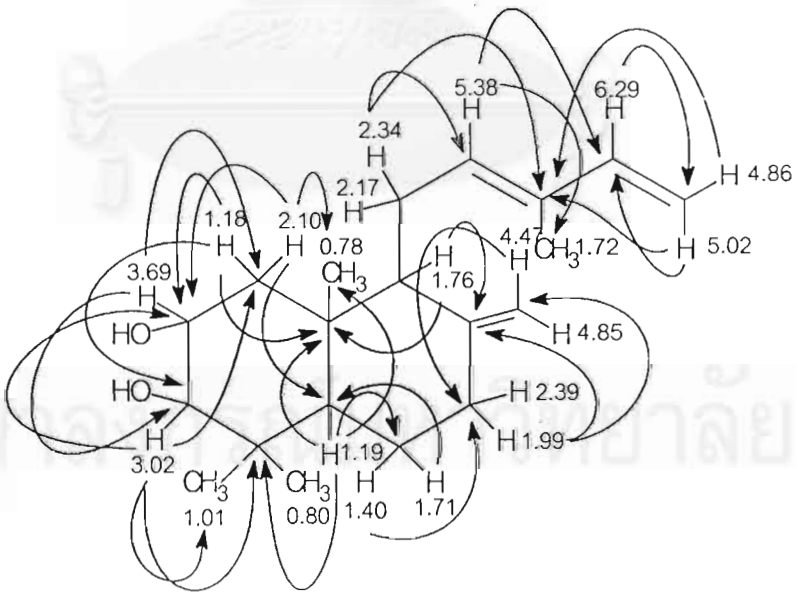


Figure 12 The HMQC correlations of compound 3

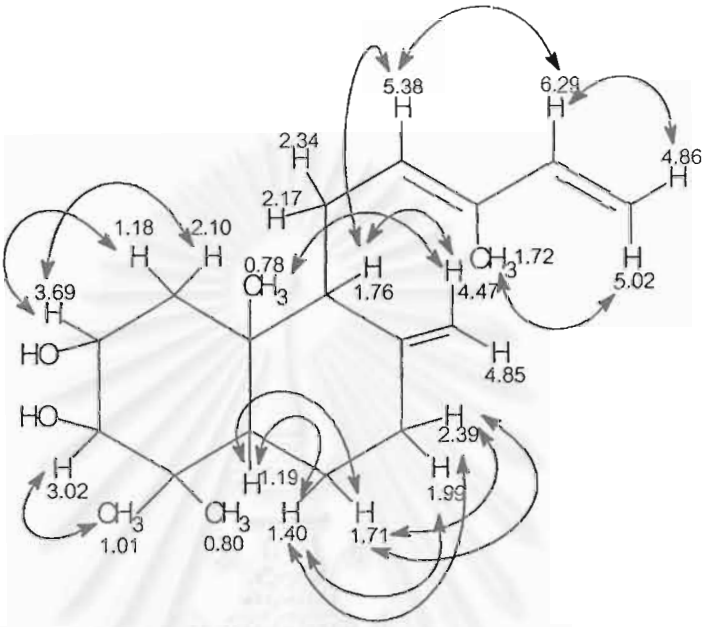


Figure 13 The COSY correlatiions of compound 3

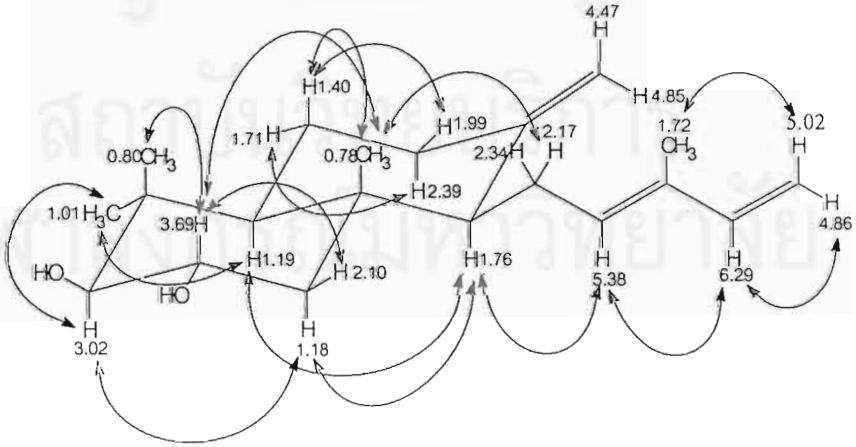


Figure 14 The NOESY correlations of compound 3

Table 8: The HMQC, HMBC, and COSYspectral data of compound 3

Position	δ_C	δ_H	HMBC	COSY
1	45.0(t)	1.18 dd (*J*=11.7, 12.5)	C-2,C-3,C-9,C-10,C-20	3.69
		2.10 dd (*J*=4.6,12.5)	C-2,C-3,C-5,C-10,C-20	3.69
2	69.1(d)	3.69 ddd (*J*=4.3,9.6,11.7)	-	3.02, 2.10, 1.20
3	83.5(d)	3.02 d (*J*=9.6)	C-1,C-2,C-4,C-18,C-19	3.69
4	39.3(s)	-	-	-
5	54.5(d)	1.19 dd (*J*=2.7, 12.5)	C-10,C-20	
6	23.6(t)	1.40 dddd (*J*=4.3, 12.5, 12.5, 12.5)	-	2.39, 2.00
		1.71 m	C-5	2.39, 2.00
7	37.7(T)	1.99 m	C-6,C-8,C-17	1.40, 1.70
		2.39 ddd (*J*=2.4, 4.0, 12.8)	C-5,C-8,C-9	
8	147.2(s)	-	-	-
9	56.7(d)	1.76 br.d (*J*=10.7)	-	4.47, 4.85
10	40.1(s)	-	-	-
11	23.3(t)	2.17 dd (*J*=6.7,11.0)	C-9,C-12	5.38
		2.34 br.dd (*J*=5.5,11.0)	C-8,C-12	
12	133.2(d)	5.38 dd (*J*=6.1, 6.1)	C-9,C-11,C-14,C-16	2.18, 2.34
13	133.7(s)	-	-	-
14	141.5(d)	6.29 dd (*J*=11.0, 17.4)	C-12,C-13,C-16	4.87, 5.02
15	110.1(t)	4.86 d (*J*=11.0)	C-13	6.29
		5.02 d (*J*=17.4)	C-13,C-14	
16	11.9(q)	1.72 d (*J*=0.9)	C-12,C-13,C-14	
17	108.6(t)	4.47 br.d (*J*=1.2)	C-7,C-9	1.76, 2.00
		4.85 br.d (*J*=1.2)	C-7,C-9	
18	28.8(q)	1.01 s	C-3,C-4,C-5,C-19	
19	16.6(q)	0.80 s	C-3,C-4,C-5,C-18	
20	15.4(q)	0.78 s	C-1,C-5,C-9,C-10	

[a]Carbon type as determined by DEPT experiments spectra : s=singlet, d=doublet, t=triplet, q=quartet. 1

4. Structure Elucidation of compound 4

The molecular formula of compound 4 was assigned as $C_{24}H_{36}O_4$ based on elemental analysis, ^1H and ^{13}C NMR and EIMS.

The EIMS spectrum of compound 4 exhibited $[M^+]$ at *m/z* 388. The ion at *m/z* at 328 and 268 were formed by loss of one and two acetic acid group, respectively.

The IR spectrum of compound 4 was summarized in Table 9.

Table 9. The IR absorption band assignment of compound 4.

Wave Number (cm^{-1})	Intensity	Tentative assignment
2979,2955,2850	Strong	CH stretching vibration of $-CH_2$, $-CH_3$
1732	strong	C=O stretching vibration
1645,1613	Medium	C=C stretching vibration
1440, 1384	Medium	$-CH_2$, $-CH_3$ bending
1255	Medium	C-O stretching vibration

Compound 4 was obtained from peracetylation of compound 3. It gave expected spectroscopic properties (IR, MS, ^1H and ^{13}C NMR). Thus, the structure of compound 4 was proposed to be 2,3-diacetoxy-labda-8(17),12 (*E*),14-triene as shown in figure 15. The long-range C-H correlations, COSY correlations, and NOESY correlations by HMBC spectrum were sumerize in figure 16-18.

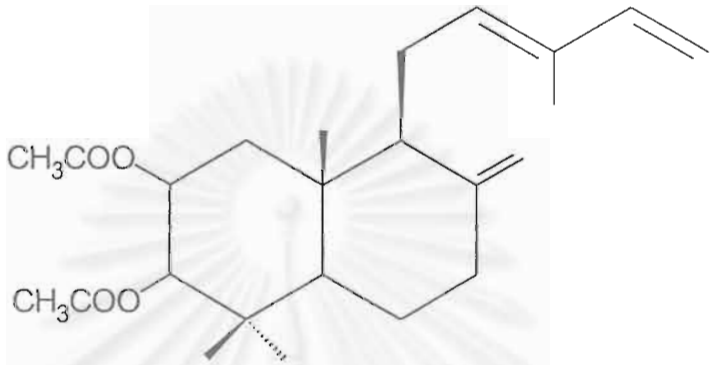


Figure 15 The structure of compound 4

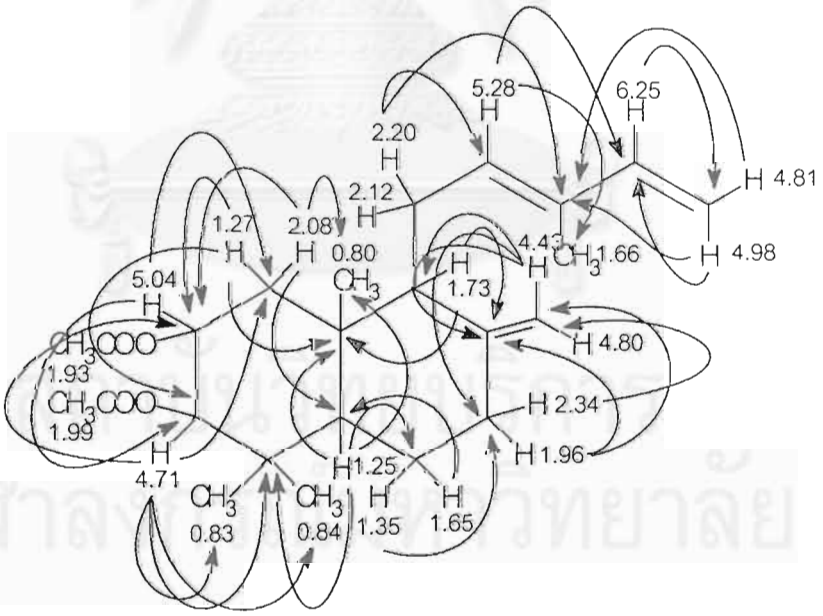


Figure 16 The HMBC correlation of compound 4

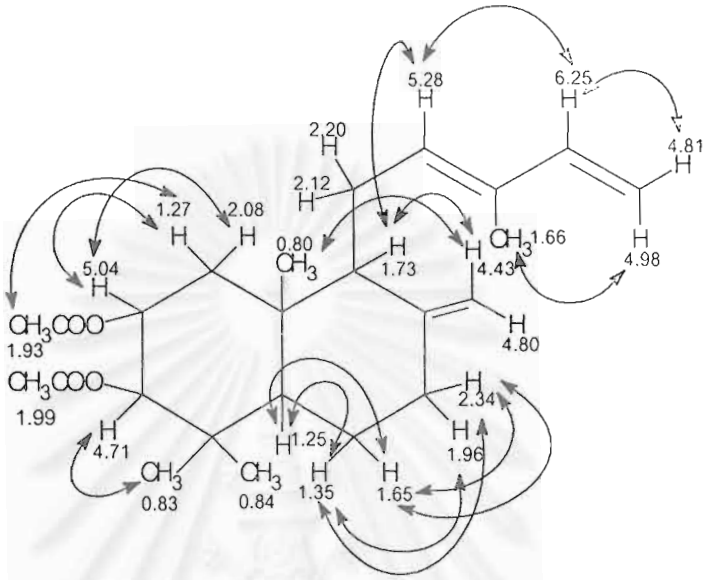


Figure 17 The COSY correlations of compound 4

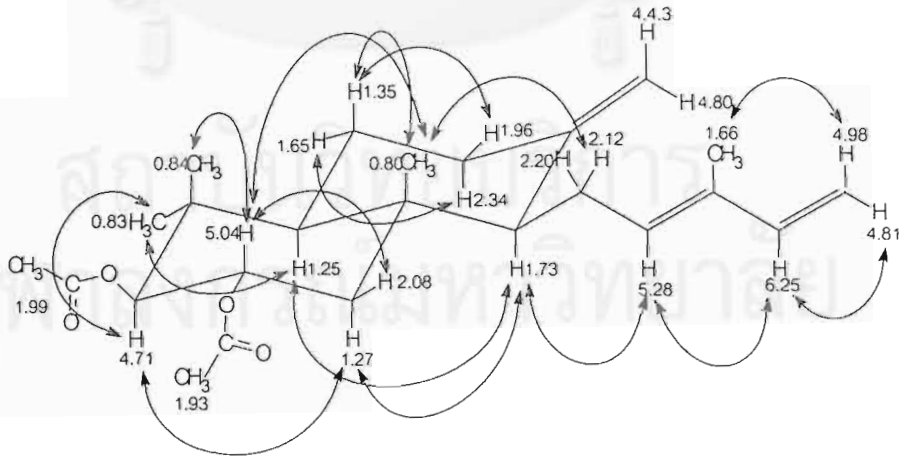


Figure 18 The NOESY correlations of compound 4

Table 10. The HMQC, HMBC, and COSYspectral data of compound 4

position	δ_C [a]	δ_H	HMBC (H to C)	COSY
1	42.4(t)	1.27 dd (=11.9, 12.3)	C-2,C-3,C-9,C-10,C-20	5.04
		2.08 dd (*J*=4.6,12.3)	C-2,C-3,C-5,C-10,C-20	5.04
2	70.0(d)	5.04 ddd (*J*=4.6,10.5,11.9)	C-1,C=O	1.27,2.08,4.71
3	80.2(d)	4.71d (*J*=10.5)	C-2,C-4,C-18,C-19,C=O	5.04
4	39.3(s)	-	-	-
5	54.1(d)	1.25 dd (*J*=2.7, 12.8)	C-4,C-6,C-10,C-20	1.35,1.65
6	23.4(t)	1.35 dddd (*J*=4.3, 12.8, 12.8, 12.8)	C-5,C-7	1.25,2.34
		1.65 m	C-7	1.25,2.34
7	37.4(t)	1.96 m	C-8	1.35,1.65
		2.34 ddd (*J*=2.1, 4.0, 13.1)	-	1.35,1.65
8	146.6(s)	-	-	-
9	56.5(d)	1.73 br.d (*J*=9.84)	C-8,C-10	2.12,2.20
10	39.9(s)	-	-	-
11	23.3(t)	2.12 m	C-8,C-9,C-12,C-13	1.73,5.28
		2.20 m	C-8,C-9,C-12,C-13	1.73,5.28
12	132.8(d)	5.28 dd (*J*=6.4, 6.4)	C-9,C-11,C-14,C-16	2.12,2.20
13	133.8(s)	-	-	-
14	141.3(d)	6.25 dd (*J*=10.7, 17.4)	C-12,C-13,C-16	4.81,4.98
15	110.1(t)	4.81 d (*J*=10.7)	C-13	6.25
		4.98 d (*J*=17.4)	C-13,C-14	6.25
16	11.9(q)	1.66 d (*J*=0.9)	C-12,C-13,C-14	
17	108.9(t)	4.43 br.d (*J*=1.2)	C-7,C-9	1.73
		4.80 m	C-7,C-9	1.73
18	28.5(q)	0.83 s	C-3,C-4,C-5,C-19	
19	17.5(q)	0.84 s	C-3,C-4,C-5,C-18	
20	15.2(q)	0.80 s	C-1,C-5,C-9,C-10	
C=O	170.3(s)	-	-	
C=O	170.5(s)	-	-	
$\underline{CH_3}CO$	21.0(q)	1.99 s	C=O	
$\underline{CH_3}CO$	20.8(q)	1.93 s	C=O	

[a]Carbon type as determined by DEPT experiments spectra : s=singlet, d=doublet, t=triplet, q= quartet

Biological activity.

Compound 1-4 were tested for their cytotoxicity against human tumor cell lines and compound 3 showed moderate activities aganst human gastric carcinoma (KATO-3, 2.2 μg/mL), colon carcinoma (SW 620, 2.7 μg/mL), breast carcinoma (BT474, 4.6 μg/mL), hepatocarcinoma (HEP-G2, 3.7 μg/mL) and lung carcinoma (BT474, 4.6 μg/mL). Compound 4 was inactive against all cell lines (>10 μg/mL). Compound 1 was active against gastric (5.7 μg/mL) and colon carcinogen (7.1 μg/mL) while compound 2 was active against gastric (3.3 μg/mL) and breast carcinogen (5.9 μg/mL). Therefore, the presence of acetyl group did not enhance cytotoxicity of compound 3. Perhaps, monoacetylation (compound 1 and 2) and diacetylation (compound 4) of compound 3 could render their ability to from hydrogen bond with certain recepter on tumor cells and made them inactive. Cytotoxicity data of compound 1-4 was show in Table 15.

Table 11 Cytotoxicity data of compound 1-4[a]

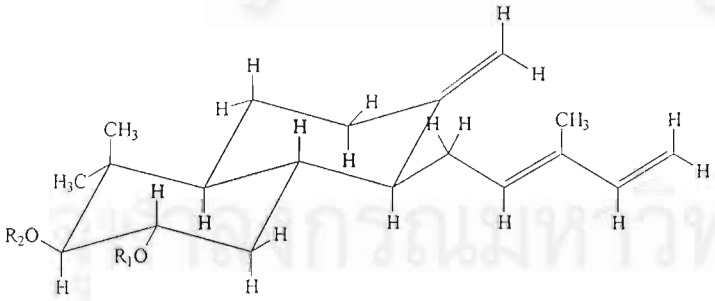
	Cell lines[b]				
Compound	KATO-3	SW 620	BT 474	HEP-G2	CHAGO
1	5.7	7.1	>10	>10	>10
2	3.3	>10	5.9	>10	>10
3	2.2	2.7	4.6	3.7	3.3
4	>10	>10	>10	>10	>10

[a] Results are expresses as IC_{50} values (μg/mL), [b] KATO-3 : Human gastric carcinoma ATCC No. HTB 103, SW 620 : Human colon carcinoma, BT 475 : Human breast carcinoma ATCC No. HTB 20, HEP-G2 : Human hepatocarcinoma ATCC No. HTB 8065, CAHGO : Human lung carcinoma

CHAPTER V

CONCLUSION

In this research, the chemical constituent of the stem bark of *Croton oblongifolius* Roxb. was investigated. The hexane crude extracted was separated on silica gel column chromatography using hexane-ethyl acetate gradient system to give three new natutal labdane diterpenoids, 3-acetoxy-labda-8(17),12(*E*),14-triene-2-ol (**1**), 2-acetoxy-labda-8(17),12(*E*),14-triene-3-ol (**2**), labda-8(17),12(*E*),14-triene-2,3-diol (**3**). Compound **4** was obtained from acetylation of compound **3**. Compound **4** was assigned as 2,3-diacetoxy-labda-8(17),12(*E*),14-triene (**4**). In this report we present a full account of the structure elucidation of **1-4** by one and two-dimentional NMR spectroscopy. The structure of these compounds were shown in the figure 19.



	R_1	R_2
1	Ac	H
2	H	Ac
3	H	H
4	Ac	Ac

Figure 19 Stereochemistry of Compound **1-4**

All isolated substances and amounts were summarized in table 12.

Table 12. Isolated substances from hexane crude extract (100g) of the stem barks of *Croton oblongifolius* Roxb. and derivative

Compound	Name of compound	Weight	%wt by wt
1	3-acetoxy-labda-8(17),12(*E*),14-triene-2-ol	0.12	0.005%
2	2-acetoxy-labda-8(17),12(*E*),14-triene-3-ol	0.06	0.003%
3	labda-8(17),12(*E*),14-triene-2,3-ol	8.94	0.413%

Compound 1-4 were tested for their cytotoxicity against human tumor cell lines found that compound 3 was active with all cell lines, compound 1 was active with KATA-3 and SW620 cell lines, compound 2 was active with KATA-3 and BT474 cell lines and compound 4 was inactive with all cell lines.

REFERENCES

1. National Identity Board. Medicinal Plants of Thailand Past and Present. Bangkok : Amarin Printing Group, 1991.
2. เต็ม สมิตินันท์. **ชื่อพันธ์ไม้แห่งประเทศไทย(ชื่อพฤกษศาสตร์ชื่อพื้นเมือง)**. พิมพ์ครั้ง ที่ 2. กรุงเทพมหานคร : หจก.ฟันนี่พับลิชชิ่ง, 2523.
3. ลีนา ผู้พัฒนพงศ์. **สมุนไพรไทย ตอนที่** 5. พิมพ์ครั้งที่1. กรุงเทพมหานคร : หจก.ชุติมาการพิมพ์, 2530.
4. สายสนม กิตติขจร. **ตำราสรรพคุณสมุนไพรยาไทยแผนโบราณ**. พิมพ์ครั้งที่ กรุงเทพมหานคร : โรงพิมพ์อักษรไทย. 2526.
5. หลวงประเสริฐวิทยาศาสตร์. **ตำราสรรพคุณยาไทย**. กรุงเทพมหานคร : โรงพิมพ์ไต้เชียง, 2548.
6. เสงี่ยม พงษ์บุญรอด. **ไม้เทศ เมืองไทย สรรพคุณของยาเทศและยาไทย**. กรุงเทพมหานคร : เกษมบรรณกิจ, 2493.
7. Blatter, E., Caius, J.F. and Mhaskar, K. S. Indian Medicinal Plants. Vol. III. 2nd ed. Delhi : Jayyed Press, 1975.
8. Rao, P.S., Sachdev, T. R. and Singh, H.B. Isolation and constitution of oblongifoliol, a new diterpeen of *Croton oblongifolius*. *Tetrahedron Letters,* 1968, 45, 4685.
9. Aiyar, V. N., Rao, P.S., Sachdev, T.R. and Seshadri, T.R., Isolation and constitution of deoxyblongifolius. *Indian J. Chem.* 1969, 7, 838.
10. Aiyar, V. N. and Seshadri, T.R., Components of *Croton oblongifolius* Roxb. III Constitution of oblongifolic acid. *Tetrahedron.* 1970, 26, 5275.
11. Aiyar, V. N. and Sachdev, T.R., Chemical components of *Croton oblongifolius* Roxb. Part V. *Indian J. Chem.* 1971, 9, 613.
12. Roengsumran, S., Achayindee, S., Petsom, A., Pudhom, K., Singtothong, P., Surachetapan C. and Vilaivan, T., *J. Nat. Product.*, 1998, 61, 652.
13. Roengsumran, S., Singtothong, P., Pudhom, K., Ngamrochanavanich, N., Petsom, A., and Chaichantipyuth. C., *J. Nat. Product.*, 1999, 61, 652.

14. ประวิทย์ สิงห์โตทอง. **เคมีและฤทธิ์ทางชีวภาพของสารประกอบไดเทอร์พีนอยด์จากเปล้าใหญ่**. วิทยานิพนธ์ปริญญาดุษฎีบัณฑิต ภาควิชาเคมี บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย, 2542

15. Roengsumran, S., Petsom, A., Sommit, D., and Vilaivan, T. Labdane diterpenoids from *Croton oblongifolius*. *J. Nat. Product.*, 1998, 61, 652.

16. Aiyar, V. N., Sachdev, T.R., 11-dehydro(-)hardwickiic acid from *Croton oblongifolius* Roxb. *Phytochemistry*. 1972, 11, 1473.

17. Paolo Mannitto. **Biosynthesis of natural products**. Lills Horwood limited. 1981.

18. Devon T.K. and Scott A. I. **Handbook of Naturally occurring compounds Volume II : Terpenes**. Academic Press. 1972.

19. Jame, D. M., Slice, S. C., and Edilberto, R. S. Antimicrobial diterpenes of *Croton sondorianus*, 1. Hardwickic and 3,4-secotrachylobanoic acid. *J. Nat. Prod.* 1991, 54, 1625.

20. Carmichael, J., Degraff, W. G., Gazdar, A. F., Minna, J. D. and Mitcheell, B. *Cancer Res.* 1987, 47, 939.

21. Twentyman, P.R. and Luscombe, M. A Study of some variables in a tetrazonium dye (MTT) based assay for cell growth and chemosensitivity. *Br. J. Cancer*. 1987, 56, 279.

22. Noma, M., Suzuki, F., Garnou, K. and Kawashima, N. Two labdane diterpenoids from Nicotiana Raimondii. *Phytochemistry*. 1982, 21, 395.

23. Bohlmann, F. and Czerson, H. Neue labdan-und pimaren-derivate aus *Palafoxia Rosea*. *Phytochemistry*. 1979, 18, 115.

24. Frank A. B. Nuclear magnetic resonance spectroscopy. Academic press, Inc. 1969

25. Garbarino, J. Chamy, M. C. and Gsmbaro, V. Labdane diterpenoids from *Nolana Rootrata*. *Phytochemistry*. 1986, 25, 2833.

APPENDICES

APPENDIX A

Spectral data of Compound 1-4

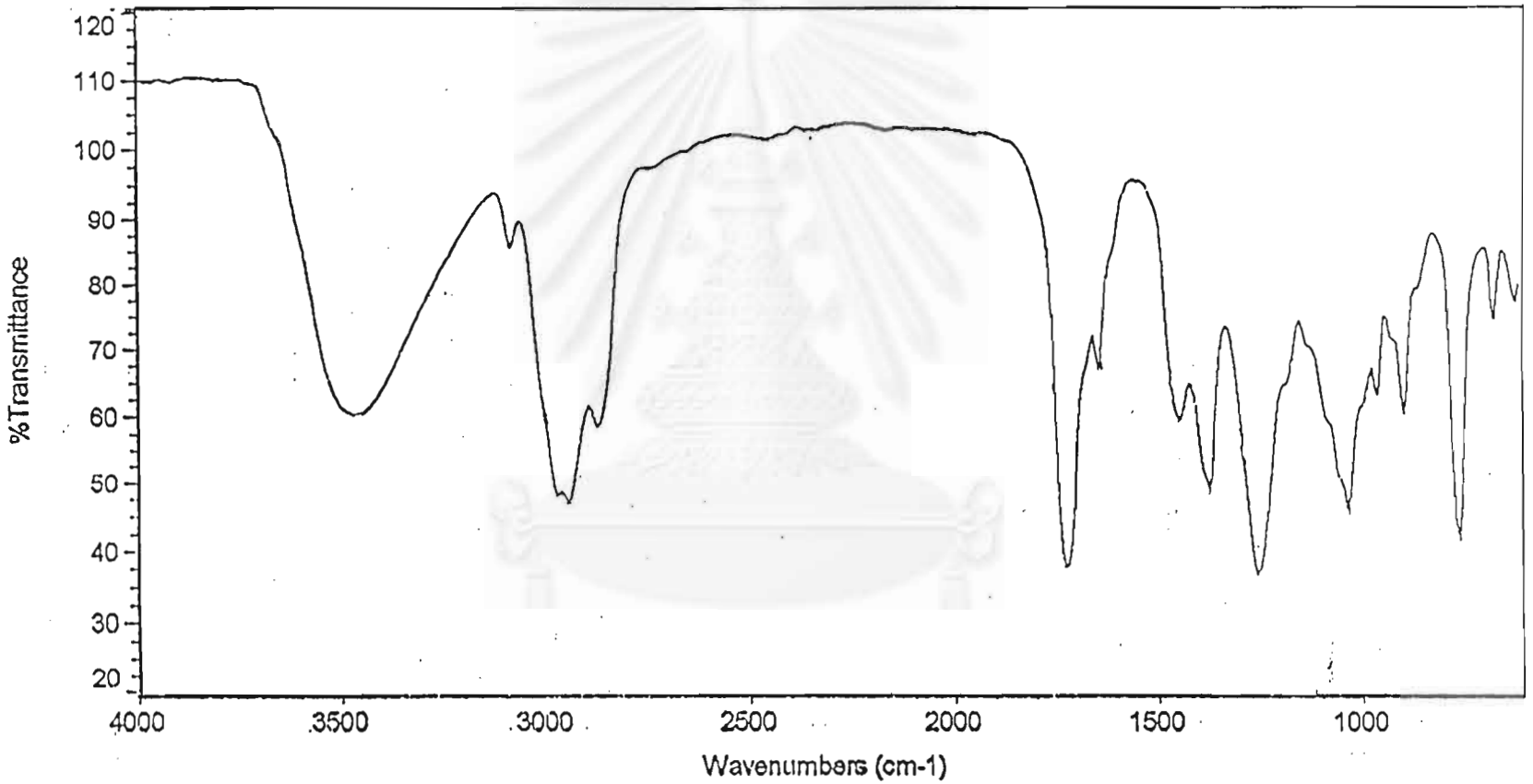


Figure 20 The IR spectrum of compound 1

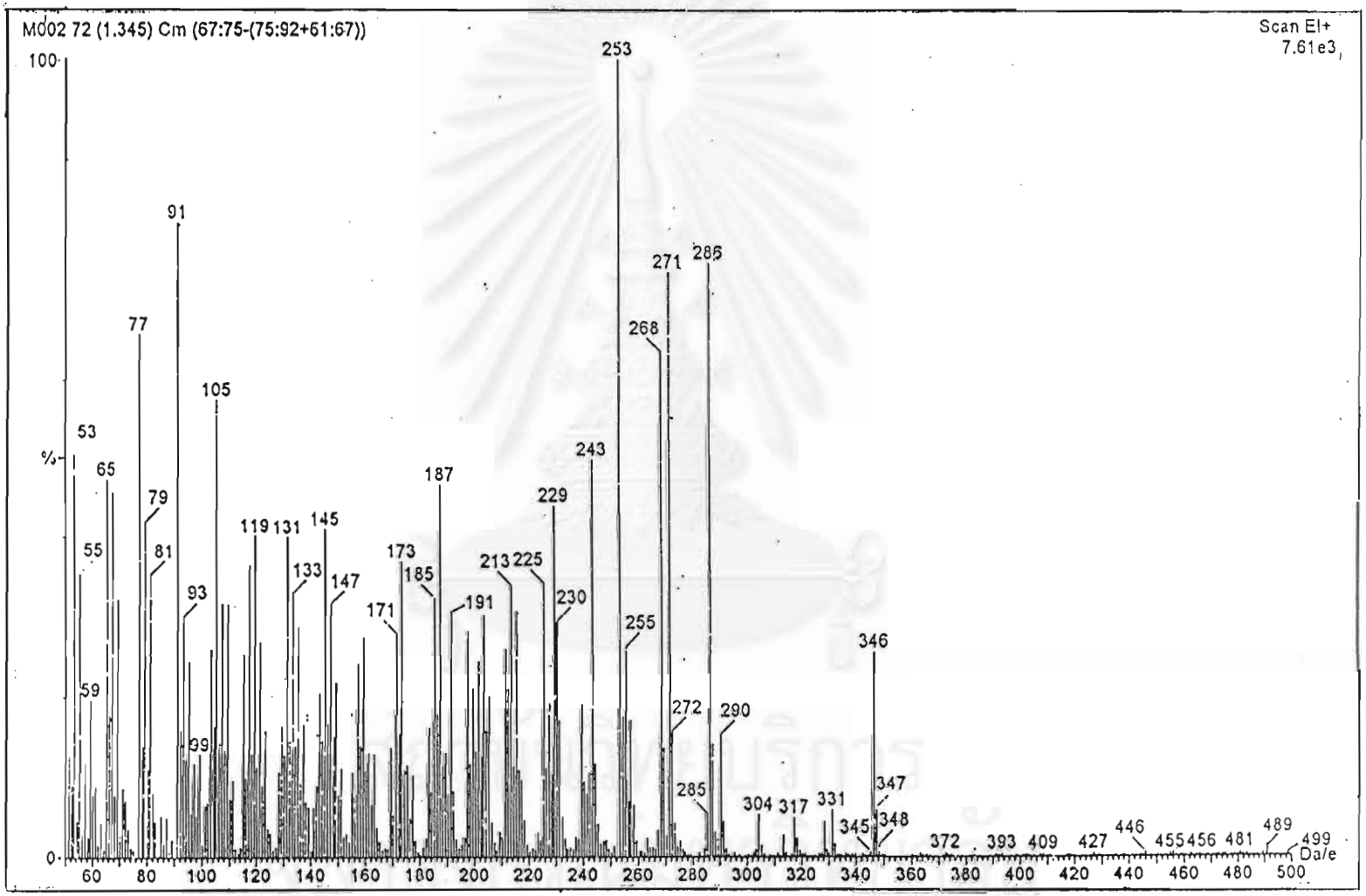


Figure 21 The EI mass spectrum of compound 1

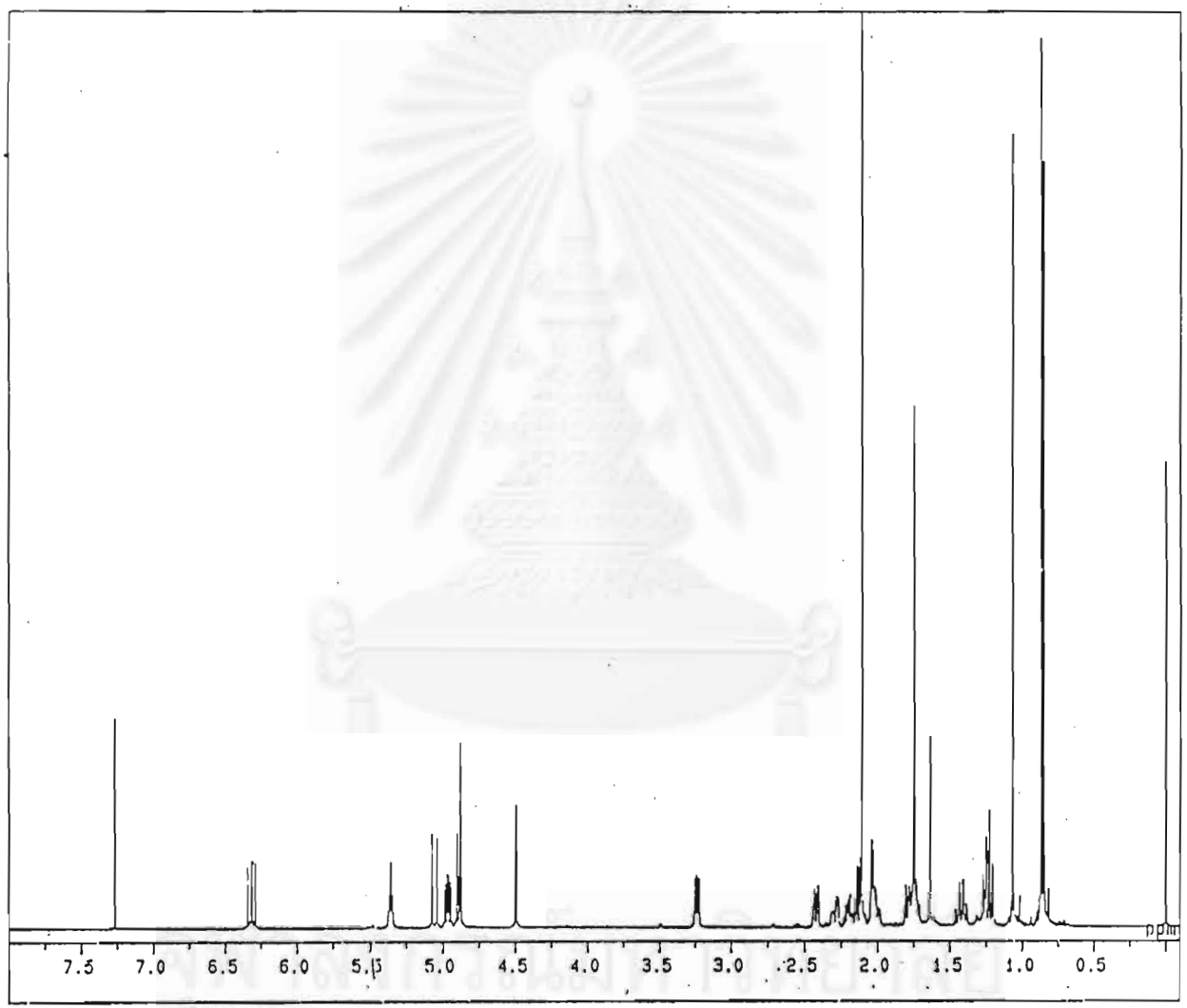


Figure 22 The ^{1}H NMR (500 MHz) spectrum of compound 1 (in $CDCl_3$)

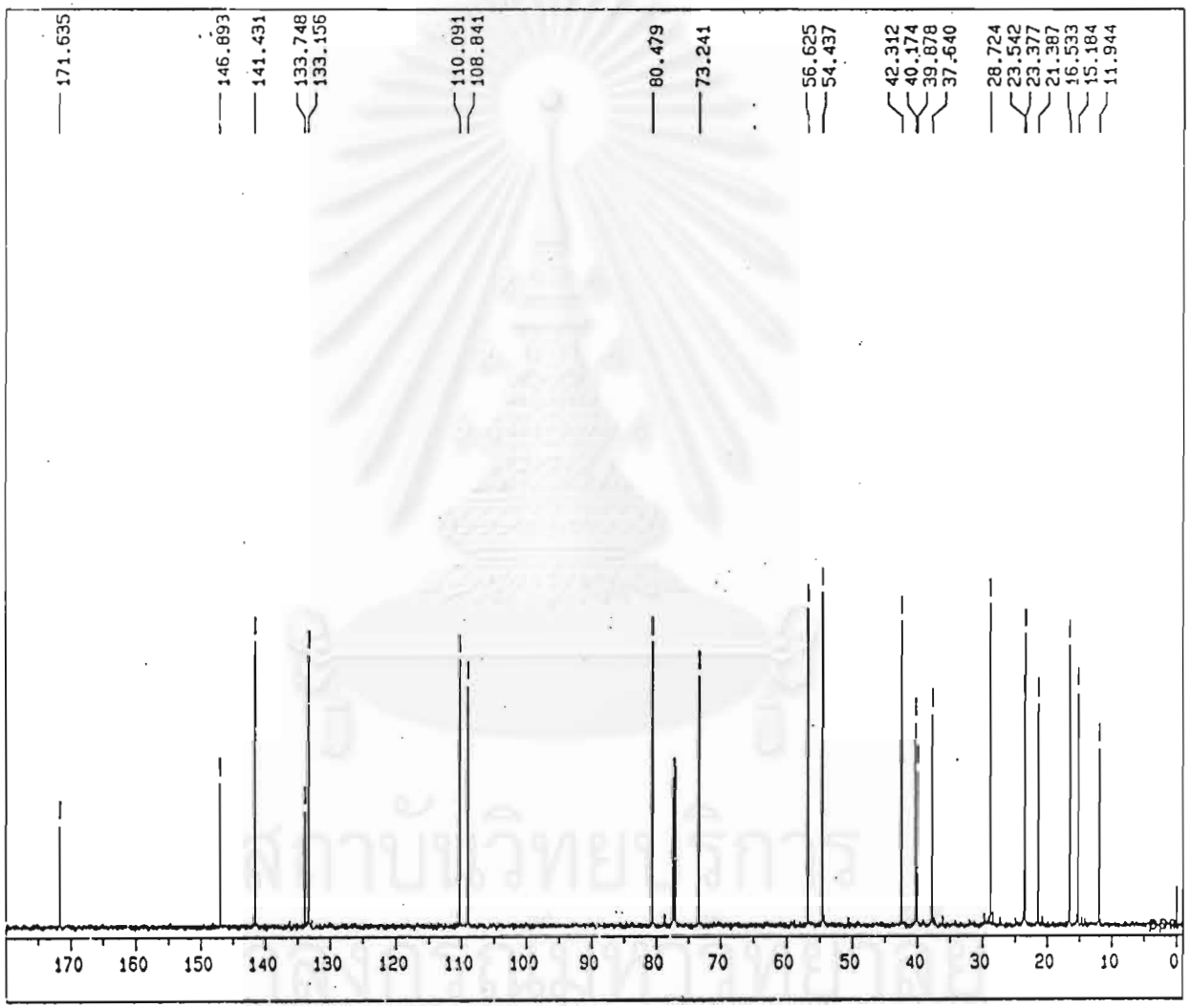


Figure 23 The ^{13}C NMR (125 MHz) spectrum of compound 1 (in $CDCl_3$)

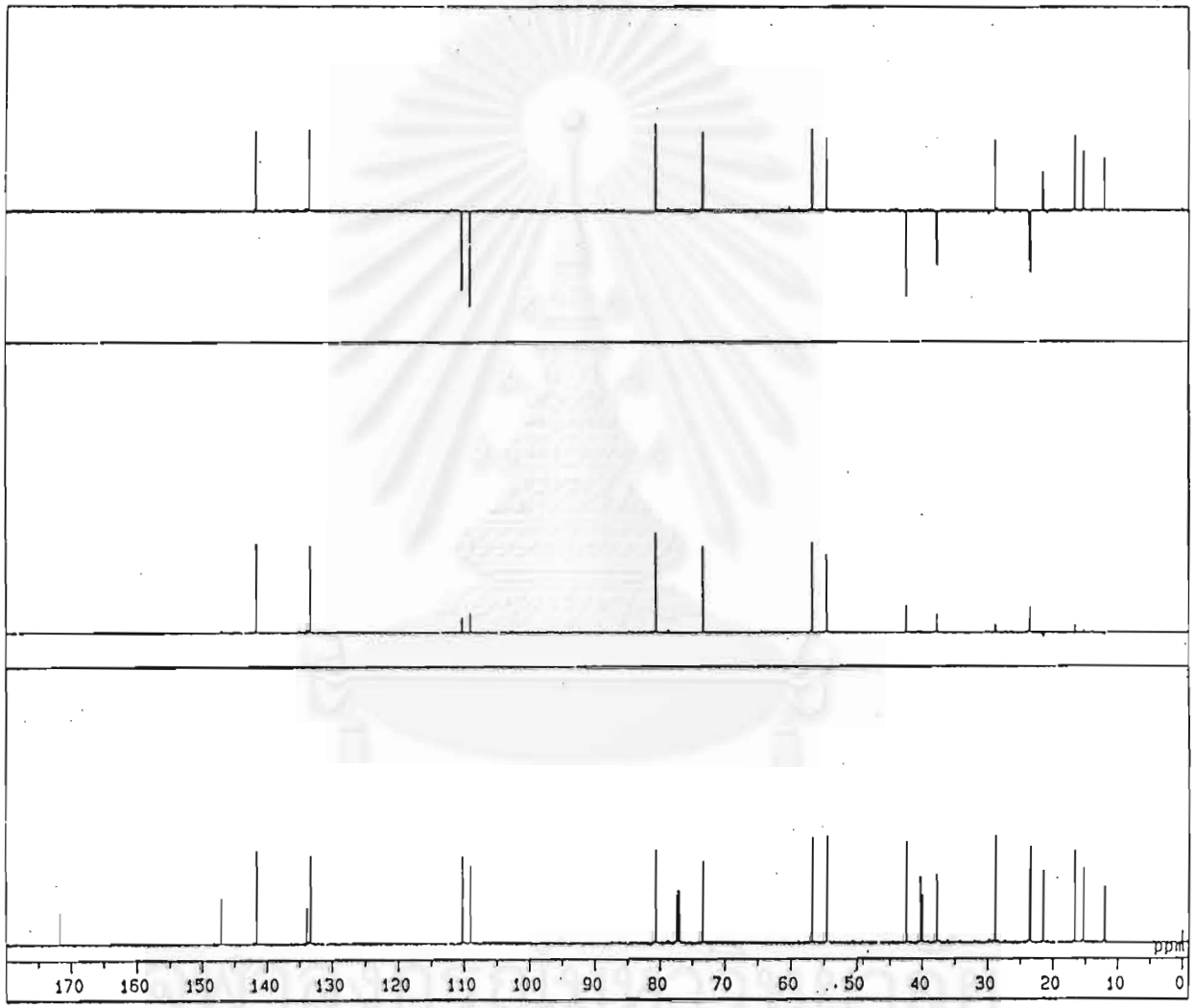


Figure 24 The DEPT (125 MHz) spectrum of compound 1 (in $CDCl_3$)

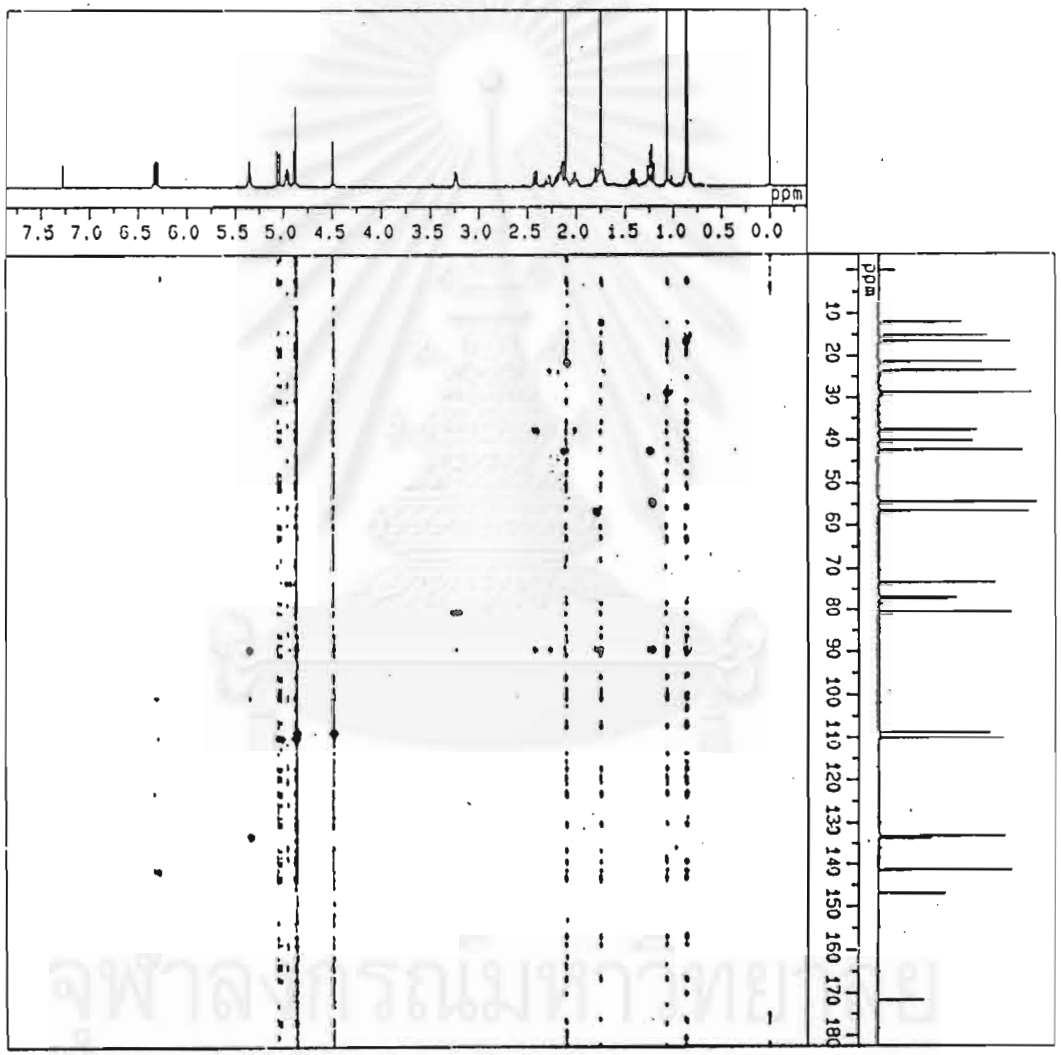


Figure 25 The HMQC (500 MHz) correlation spectrum of compound 1

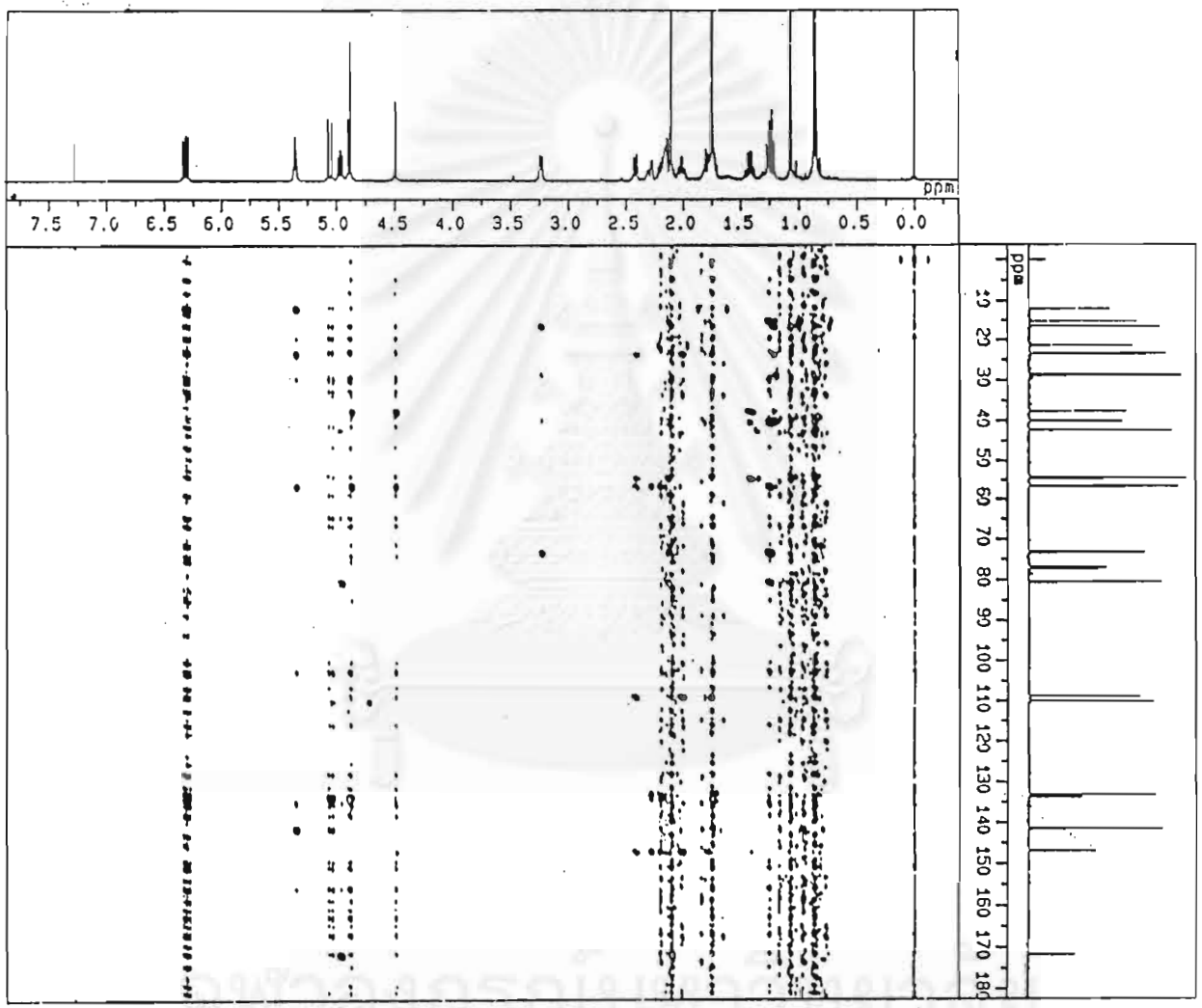


Figure 26 The HMBC (500 MHz) correlation spectrum of compound 1

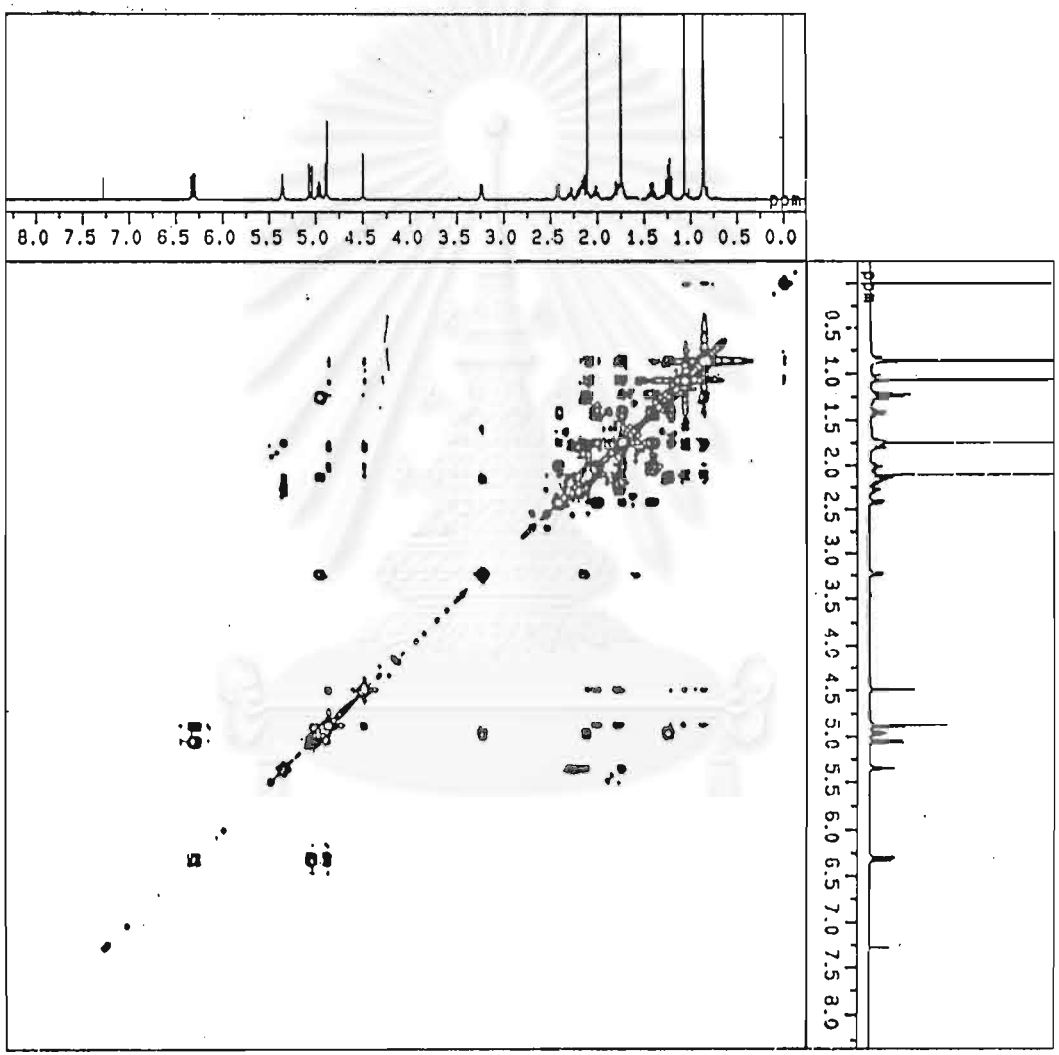


Figure 27 The COSY (500 MHz) spectrum of compound 1

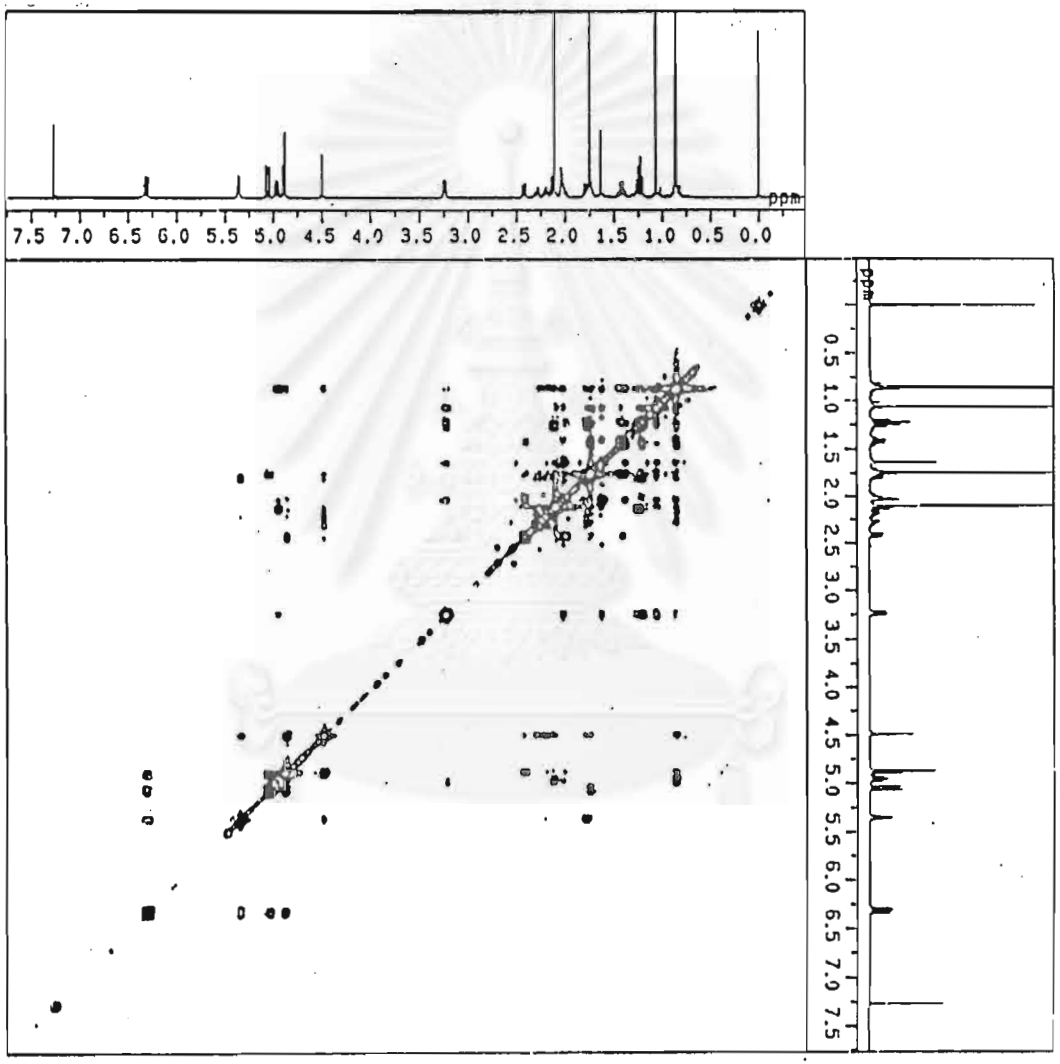


Figure 28 The NOESY (500 MHz) spectrum of compound 1

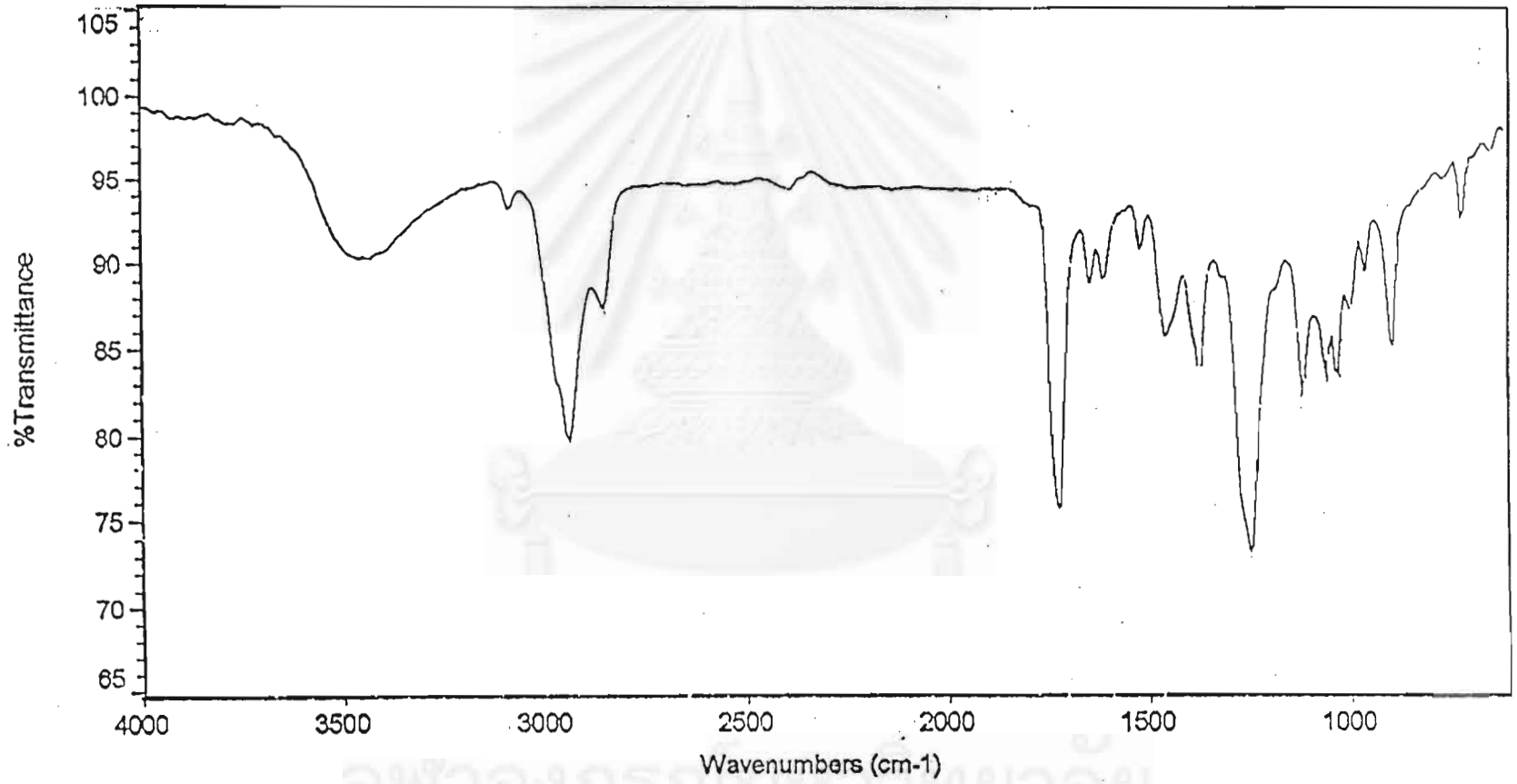


Figure 29 The IR spectrum of compound 2

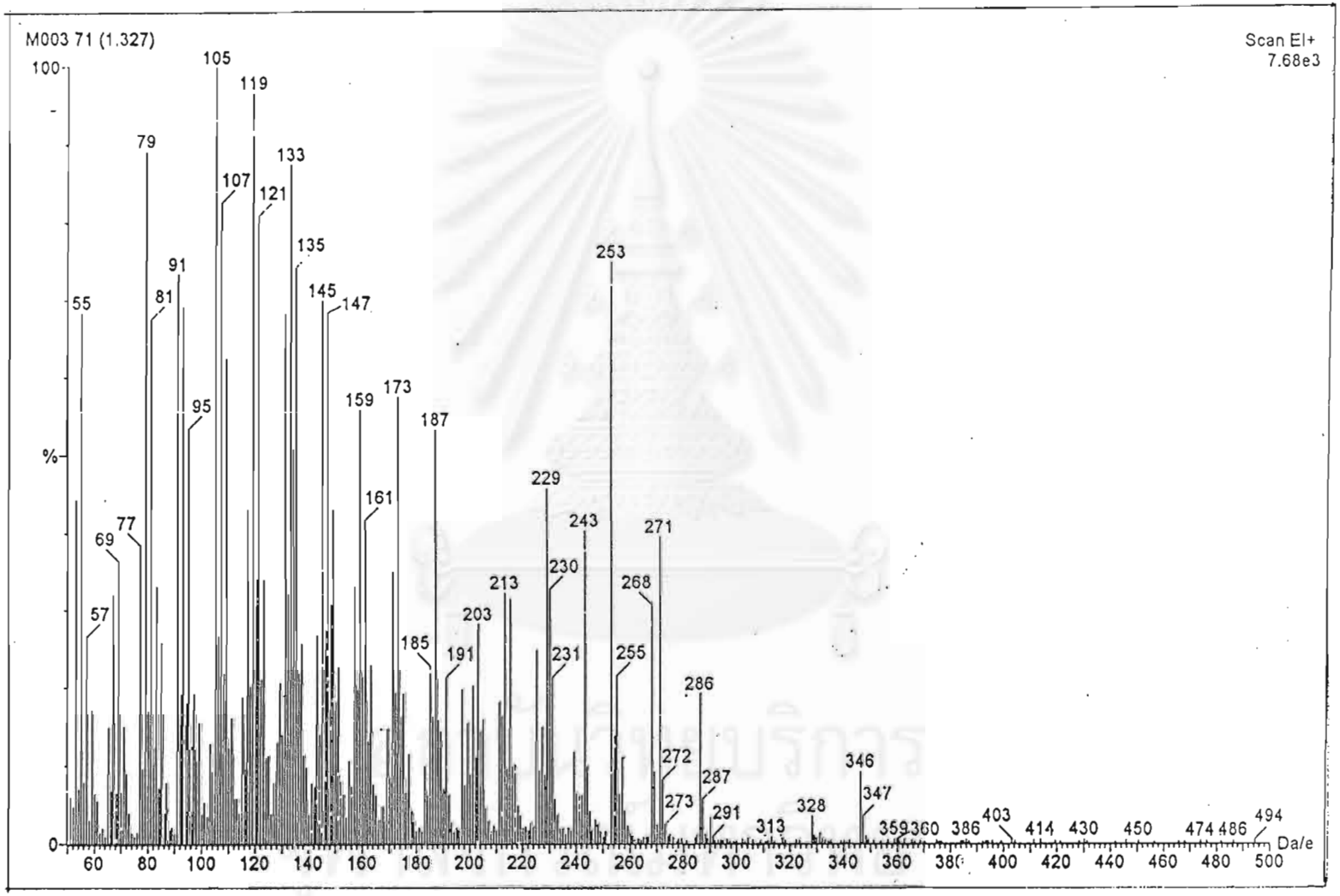


Figure 30 The EI mass spectrum of compound 2

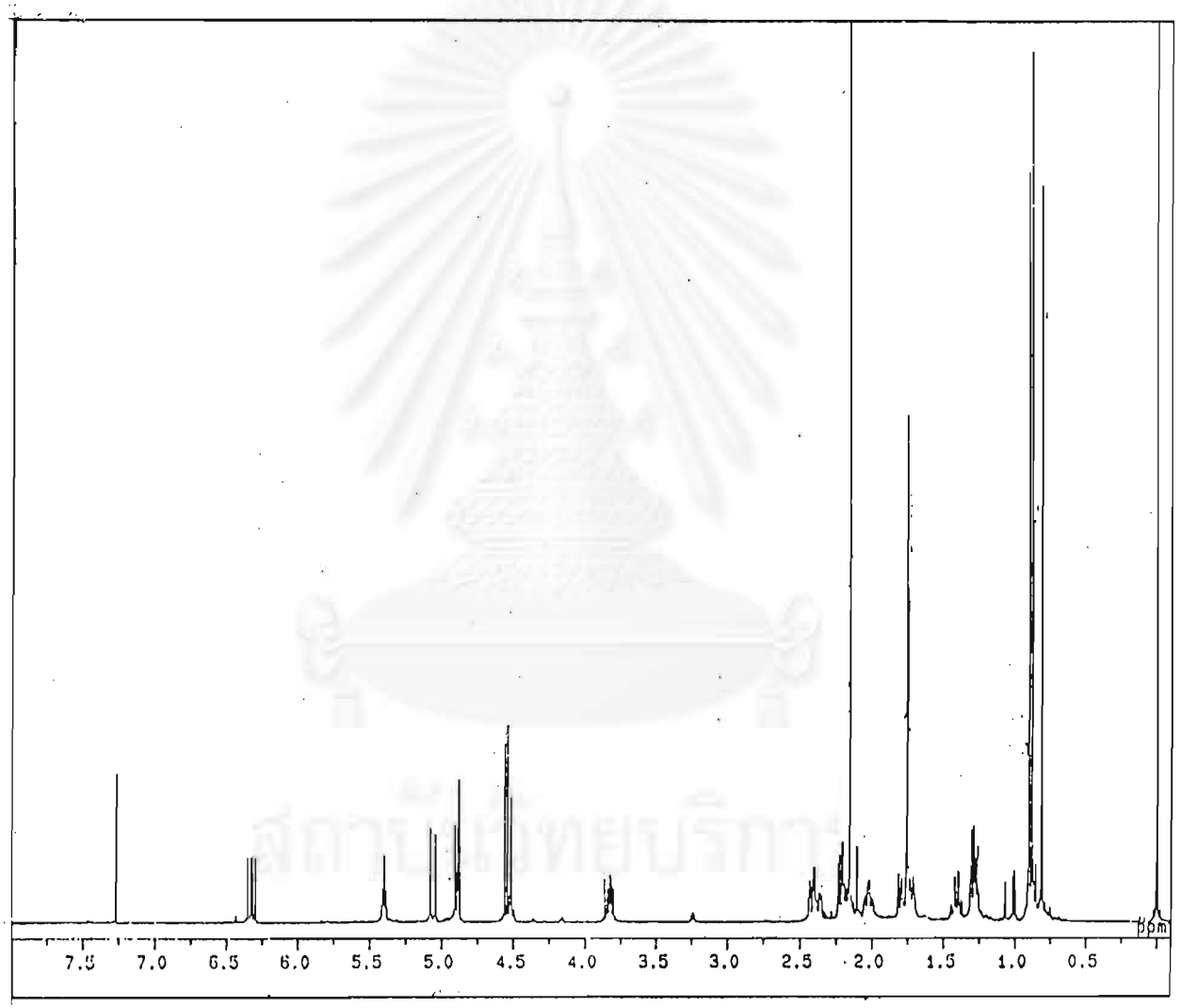


Figure 31 The ^{1}H NMR (500 MHz) spectrum of compound 2 (in $CDCl_3$)

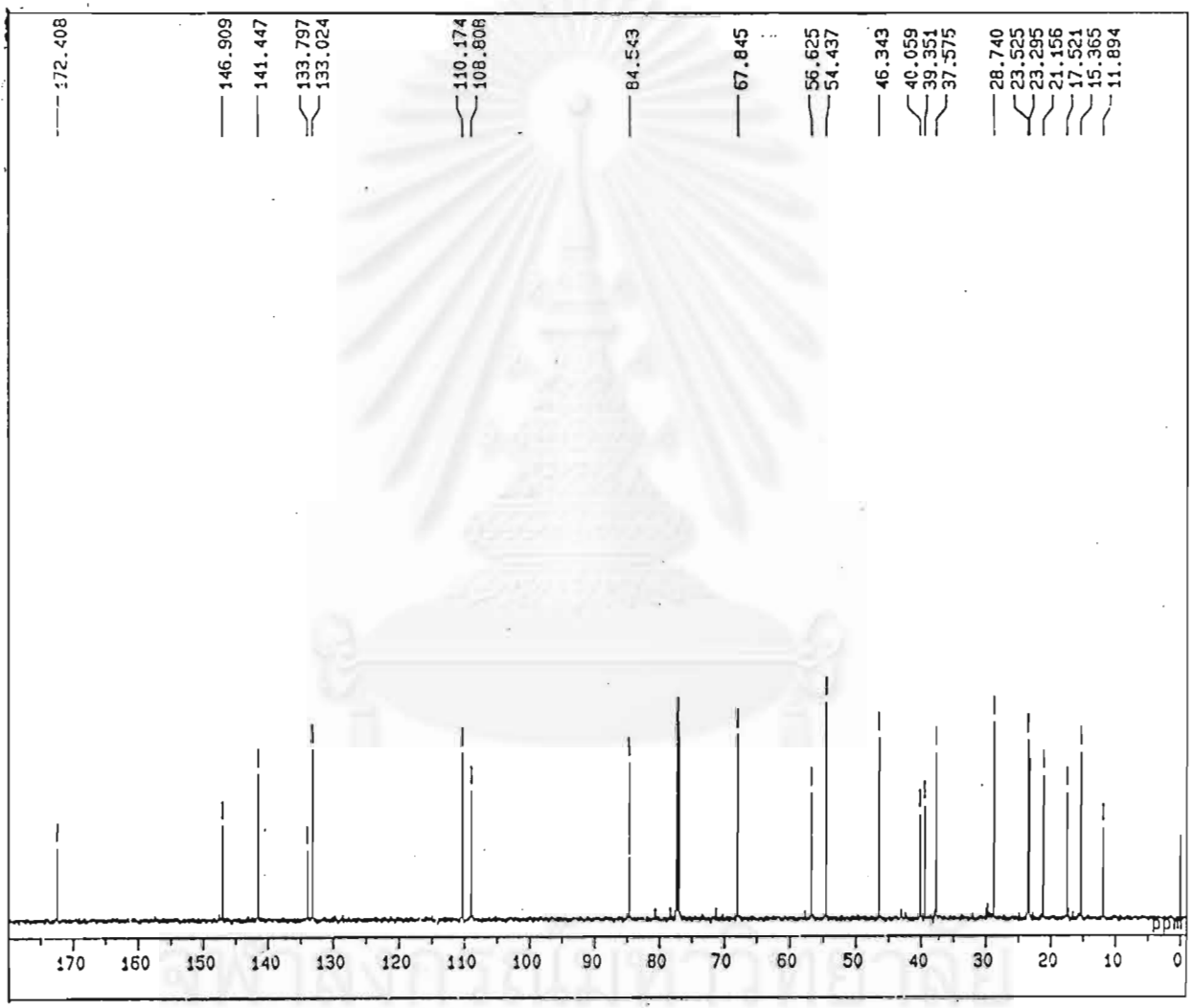


Figure 32 The ^{13}C NMR (125 MHz) spectrum of compound 2 (in $CDCl_3$)

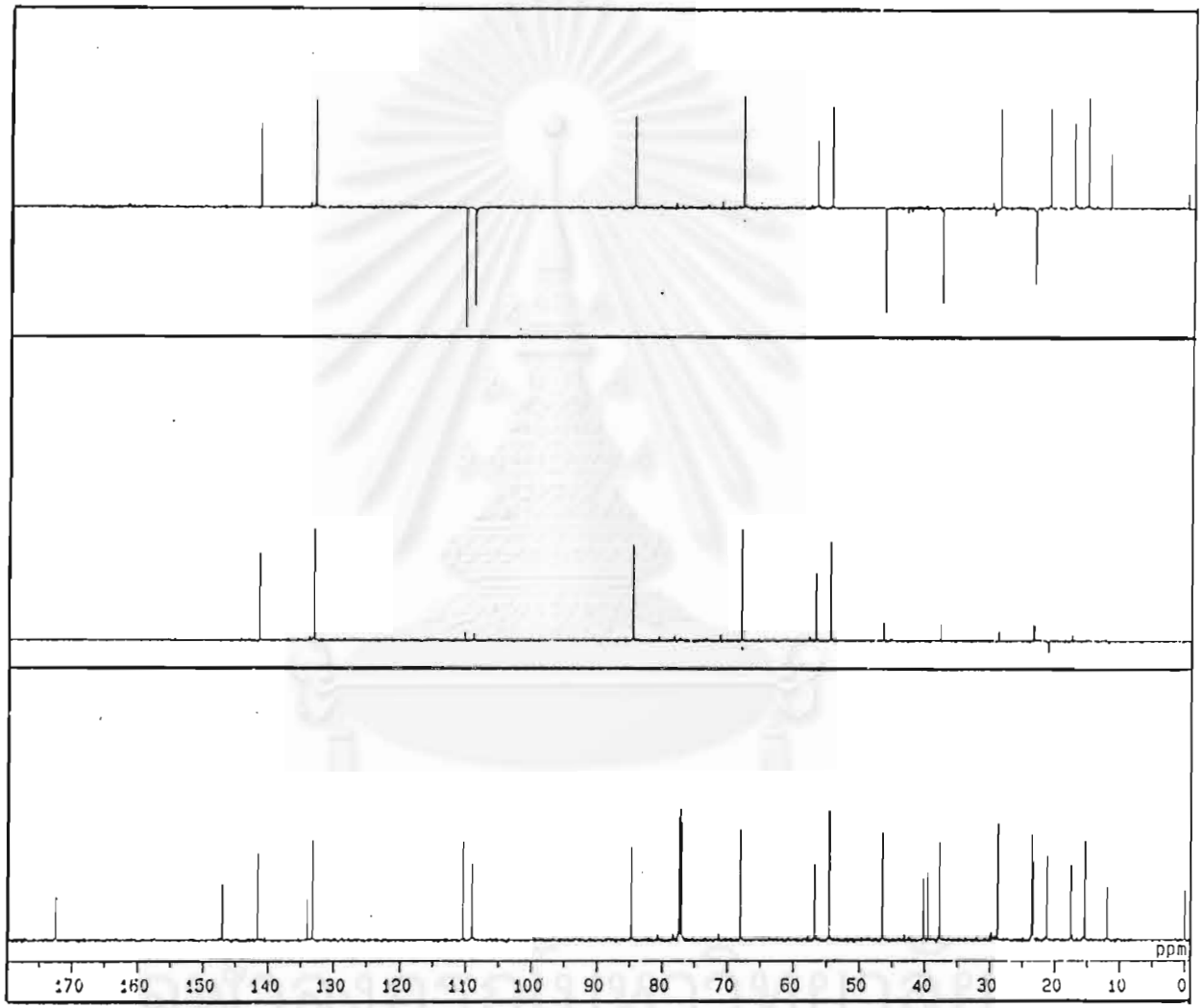


Figure 33 The DEPT (125 MHz) spectrum of compound 2 (in $CDCl_3$)

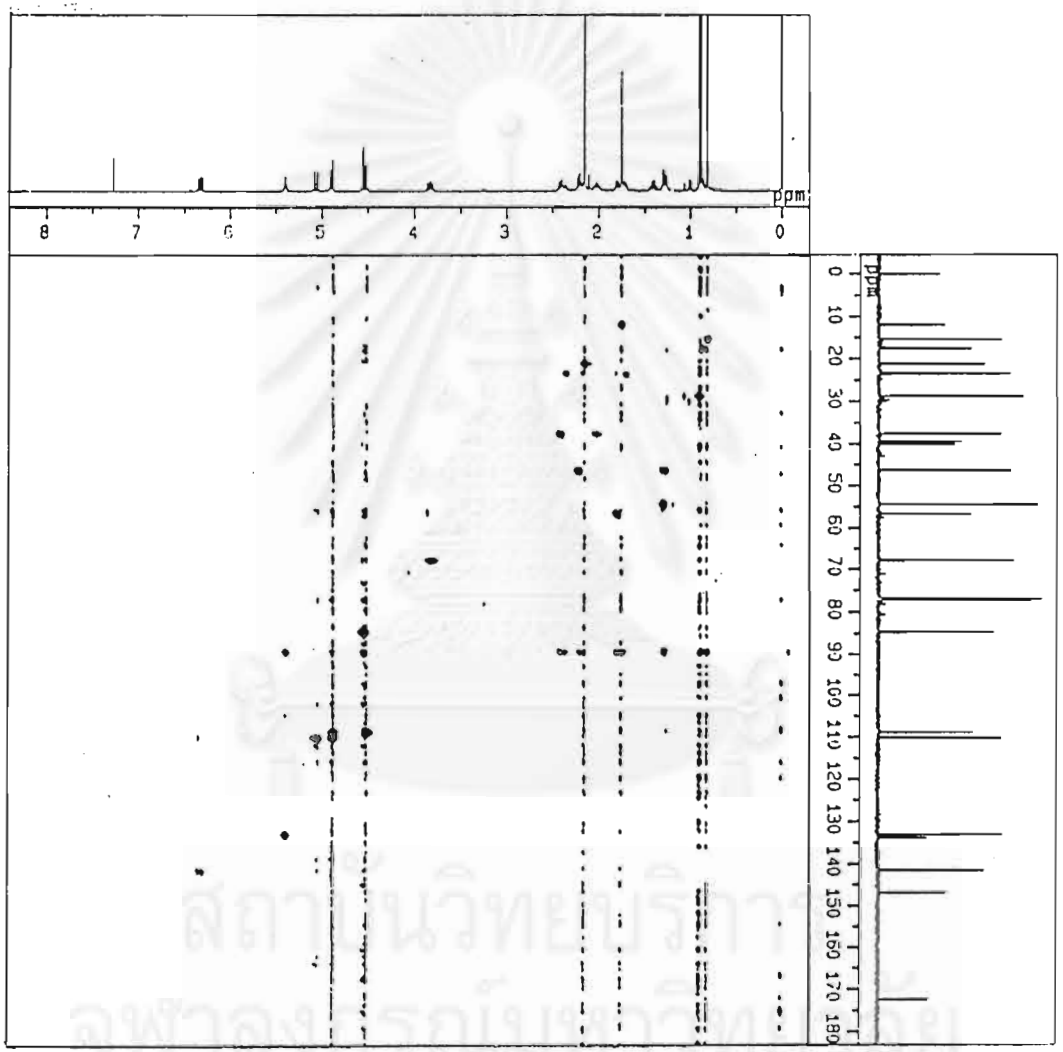


Figure 34 The HMQC (500 MHz) correlation spectrum of compound 2

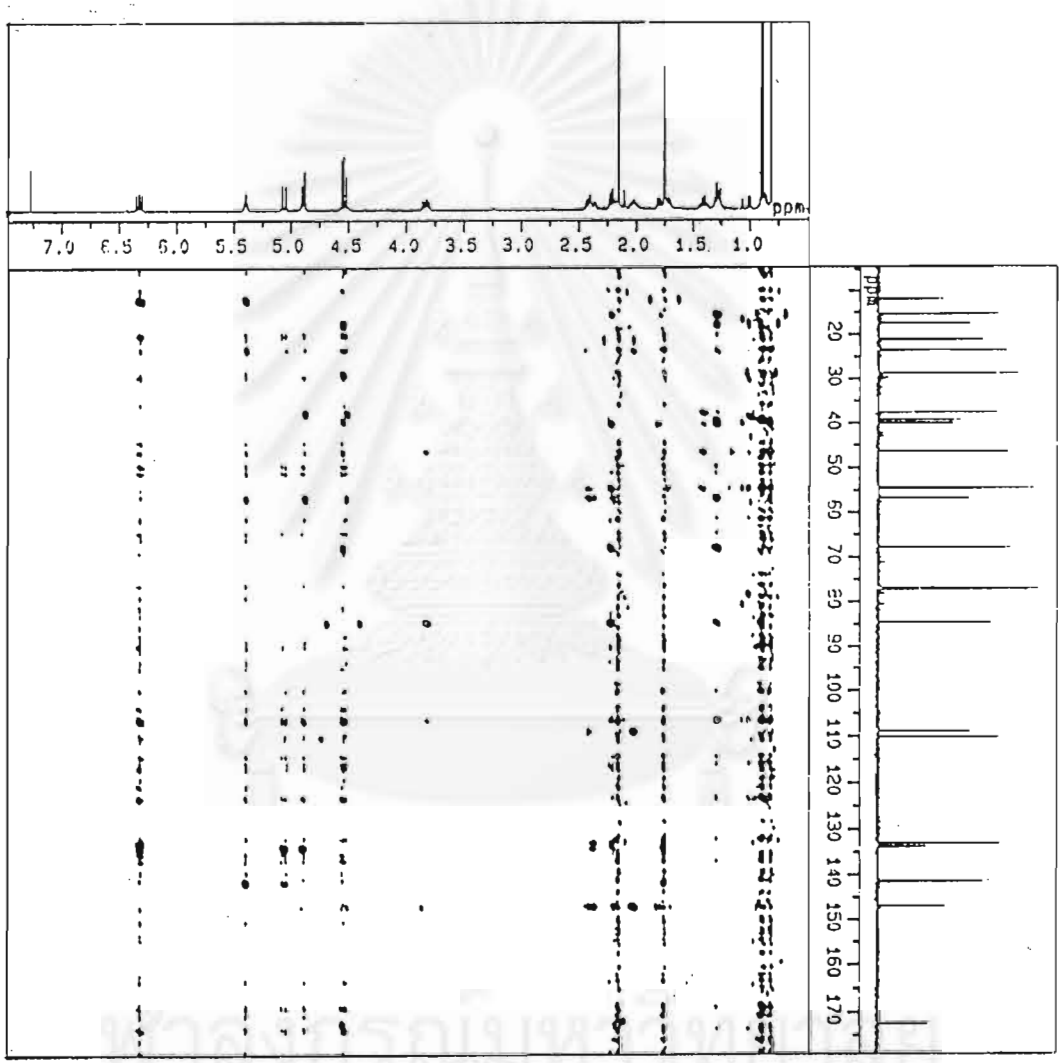


Figure 35 The HMBC (500 MHz) correlation spectrum of compound 2

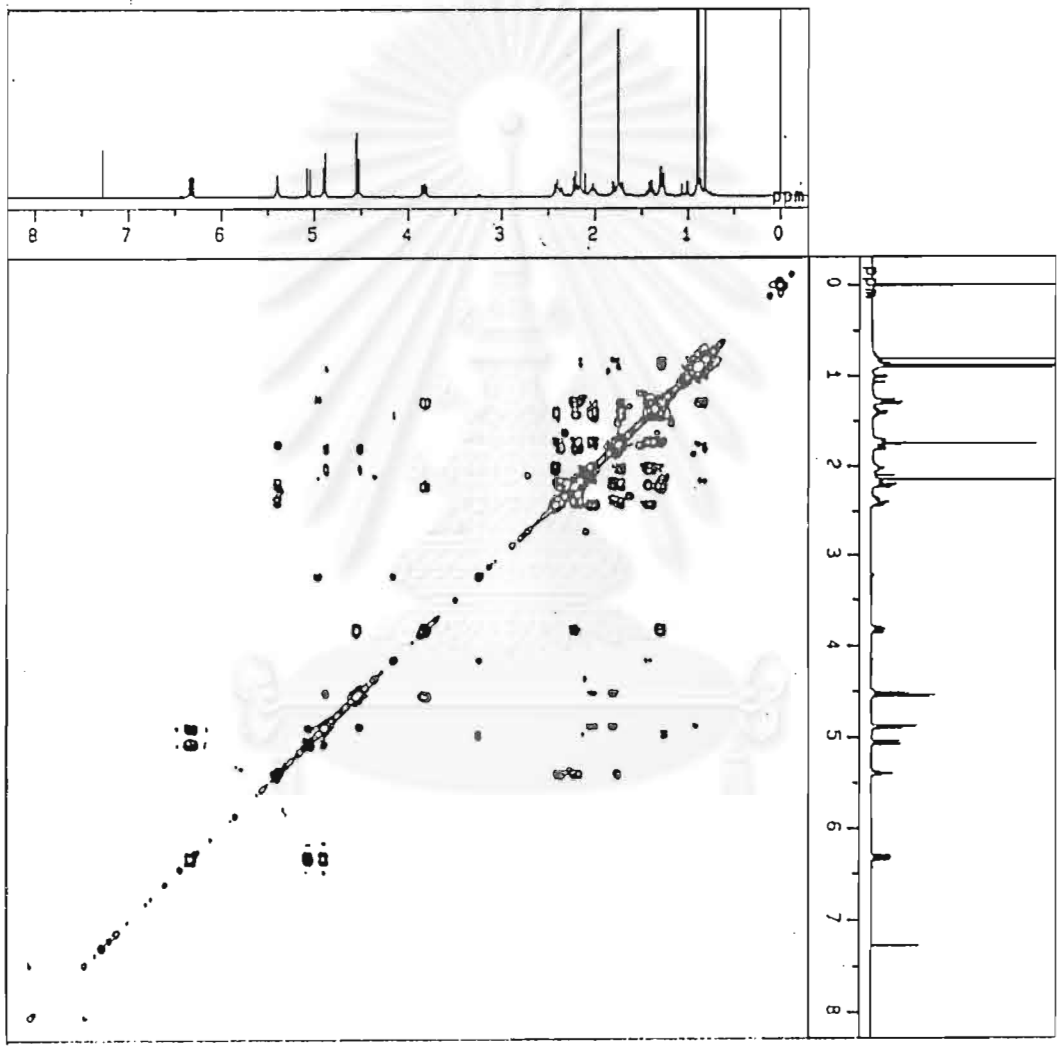


Figure 36 The COSY (500 MHz) spectrum of compound 2

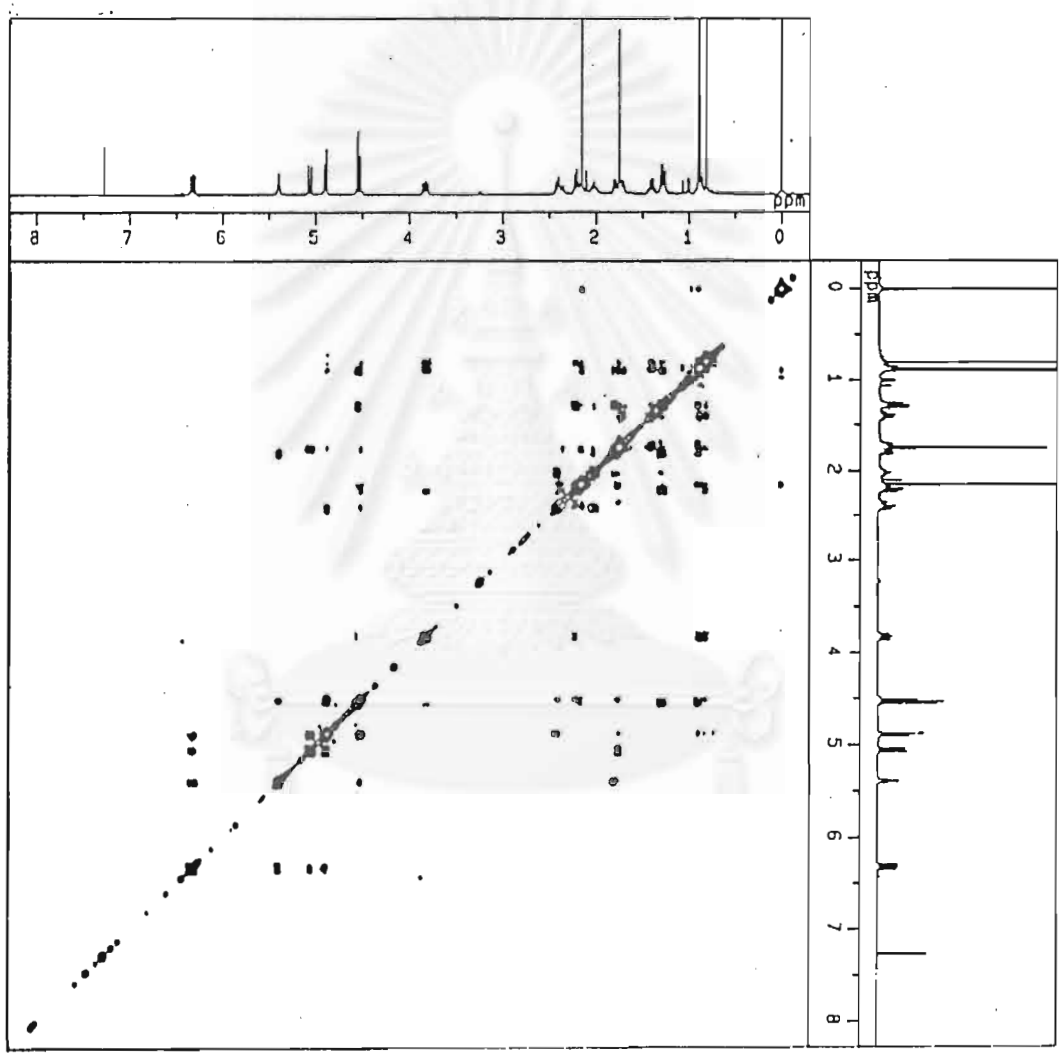


Figure 37 The NOESY (500 MHz) spectrum of compound 2

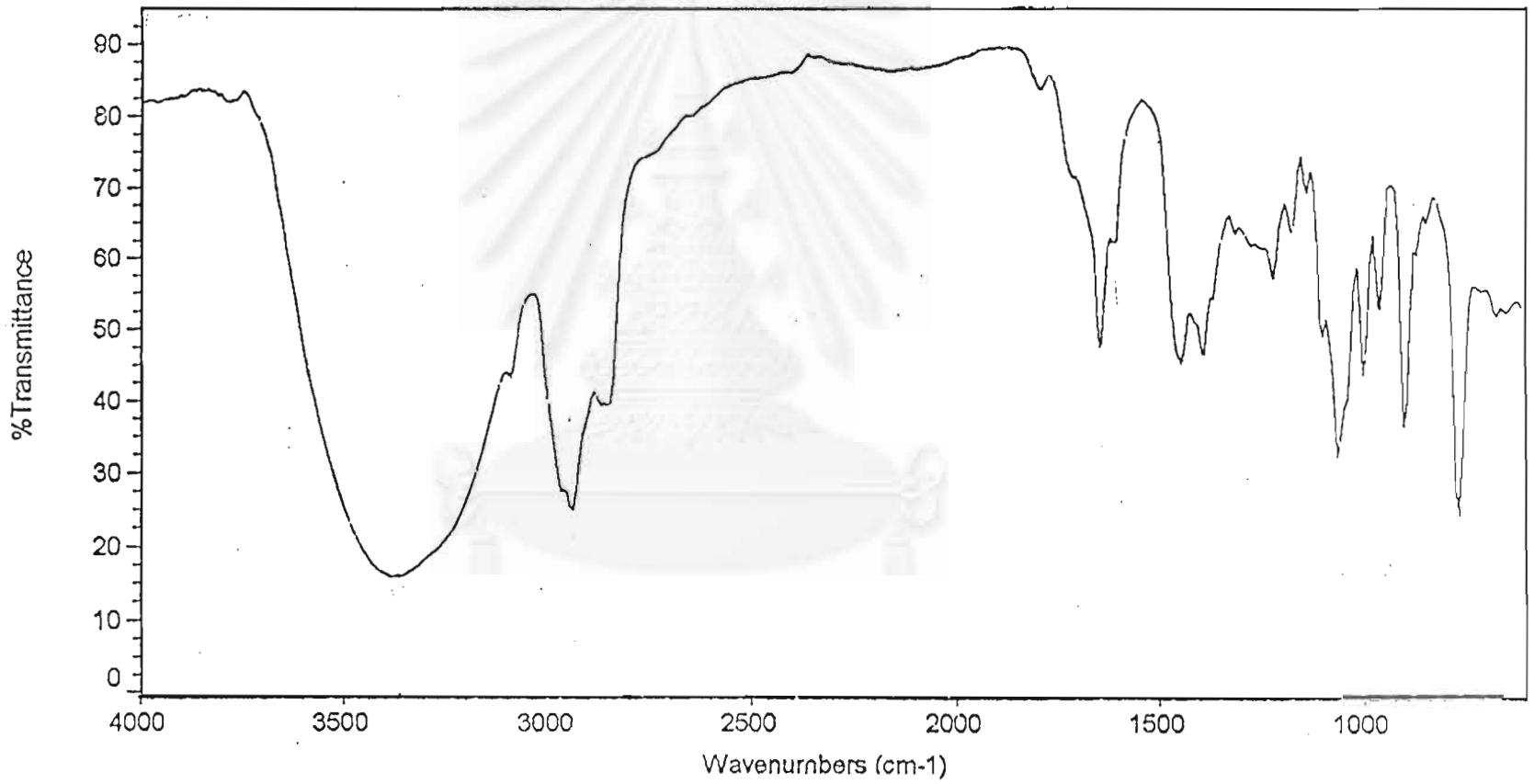


Figure 38 The IR spectrum of compound 3

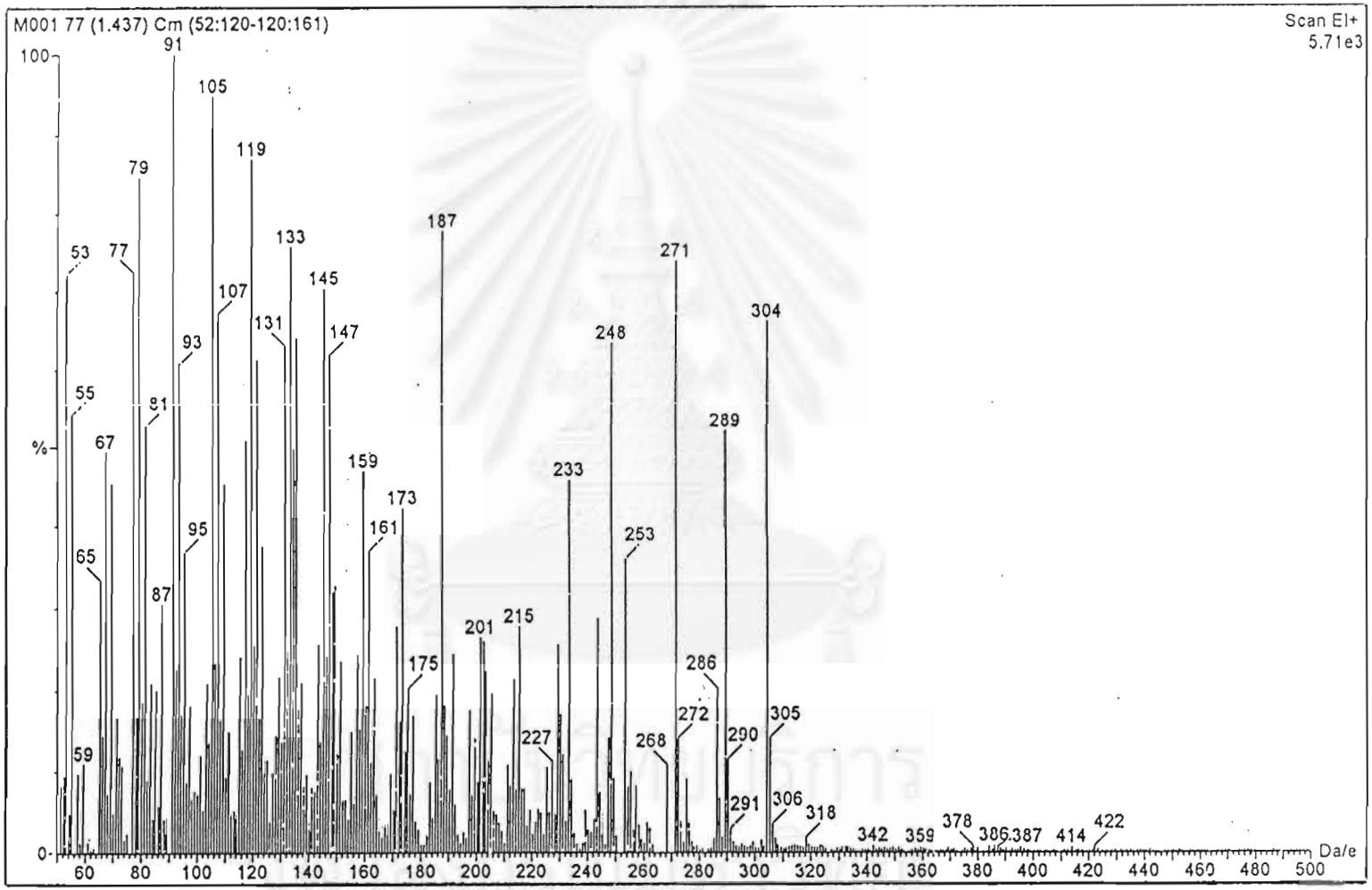


Figure 39 The EI mass spectrum of compound 3

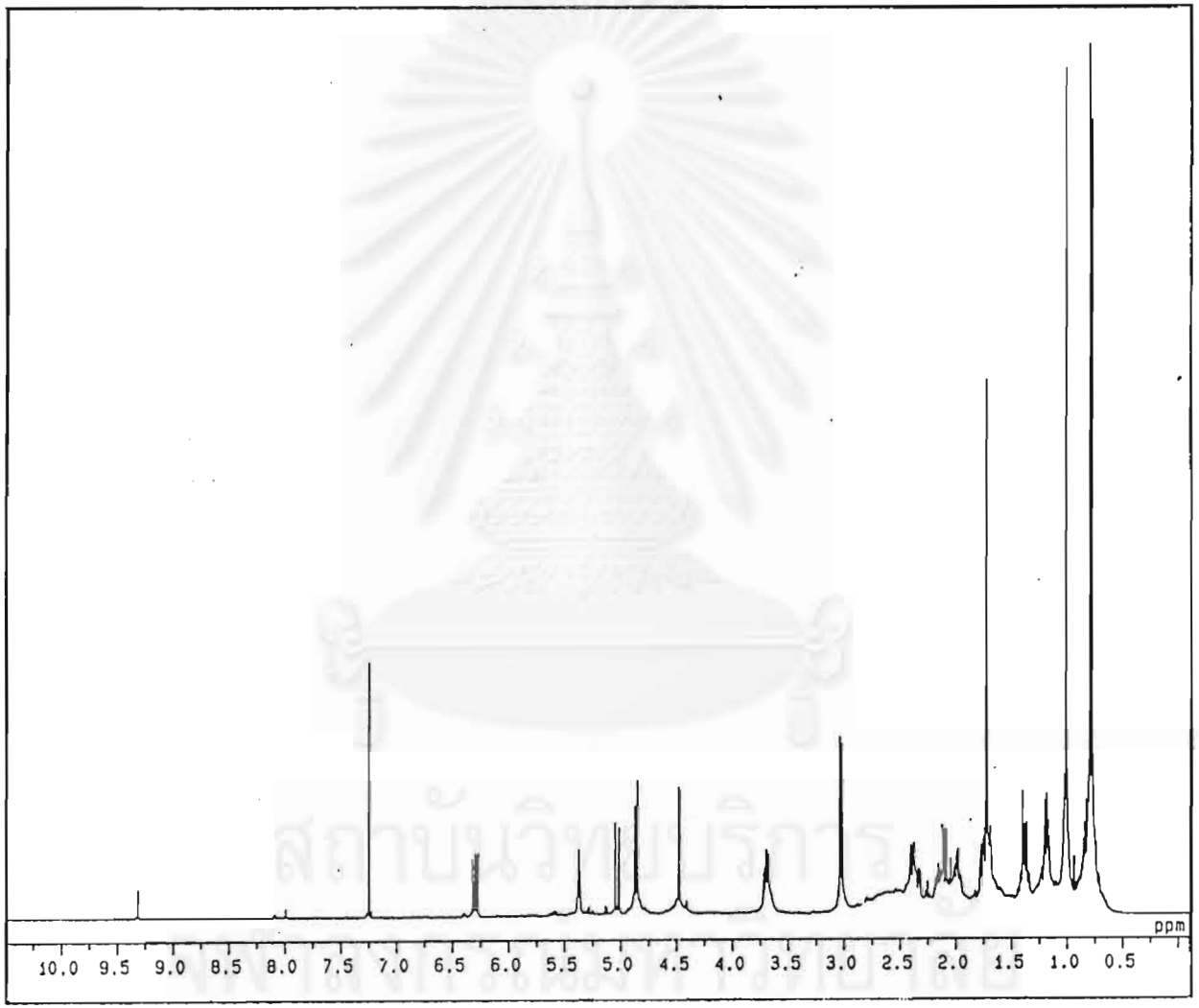


Figure 40 The ^{1}H NMR (500 MHz) spectrum of compound 3 (in $CDCl_3$)

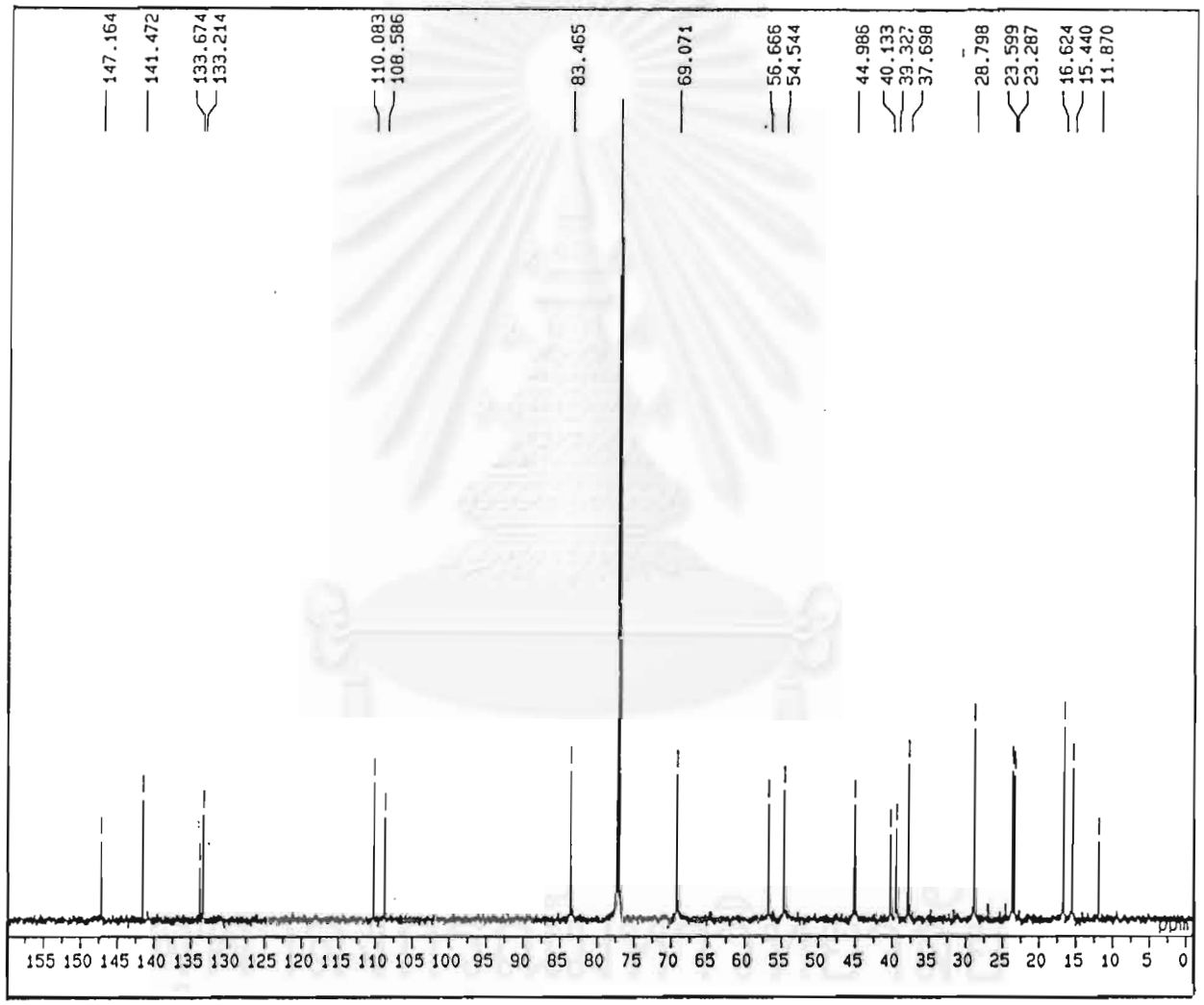


Figure 41 .The ^{13}C NMR (125 MHz) spectrum of compound 3 (in $CDCl_3$)

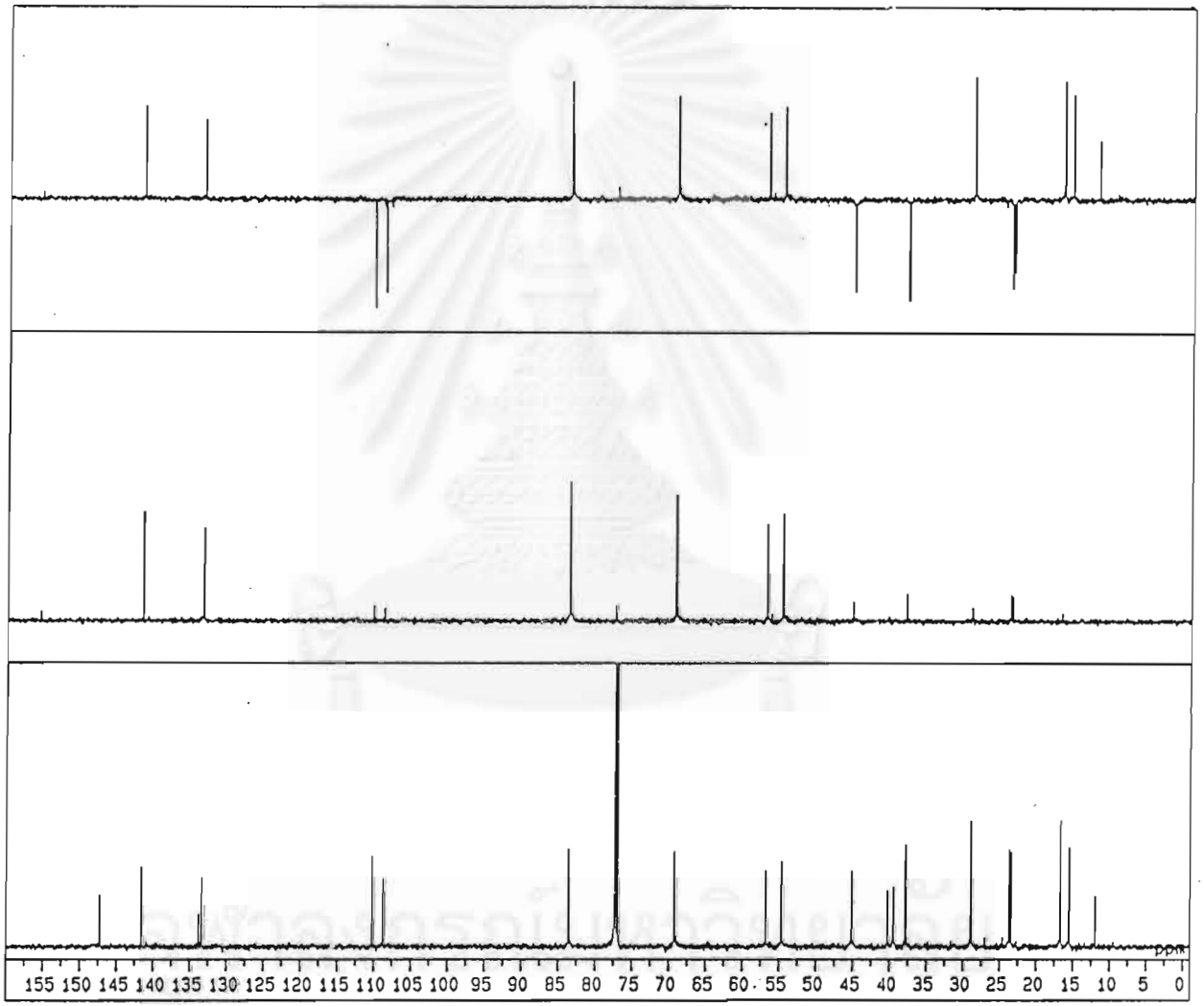


Figure 42 The DEPT (125 MHz) spectrum of compound 3 (in $CDCl_3$)

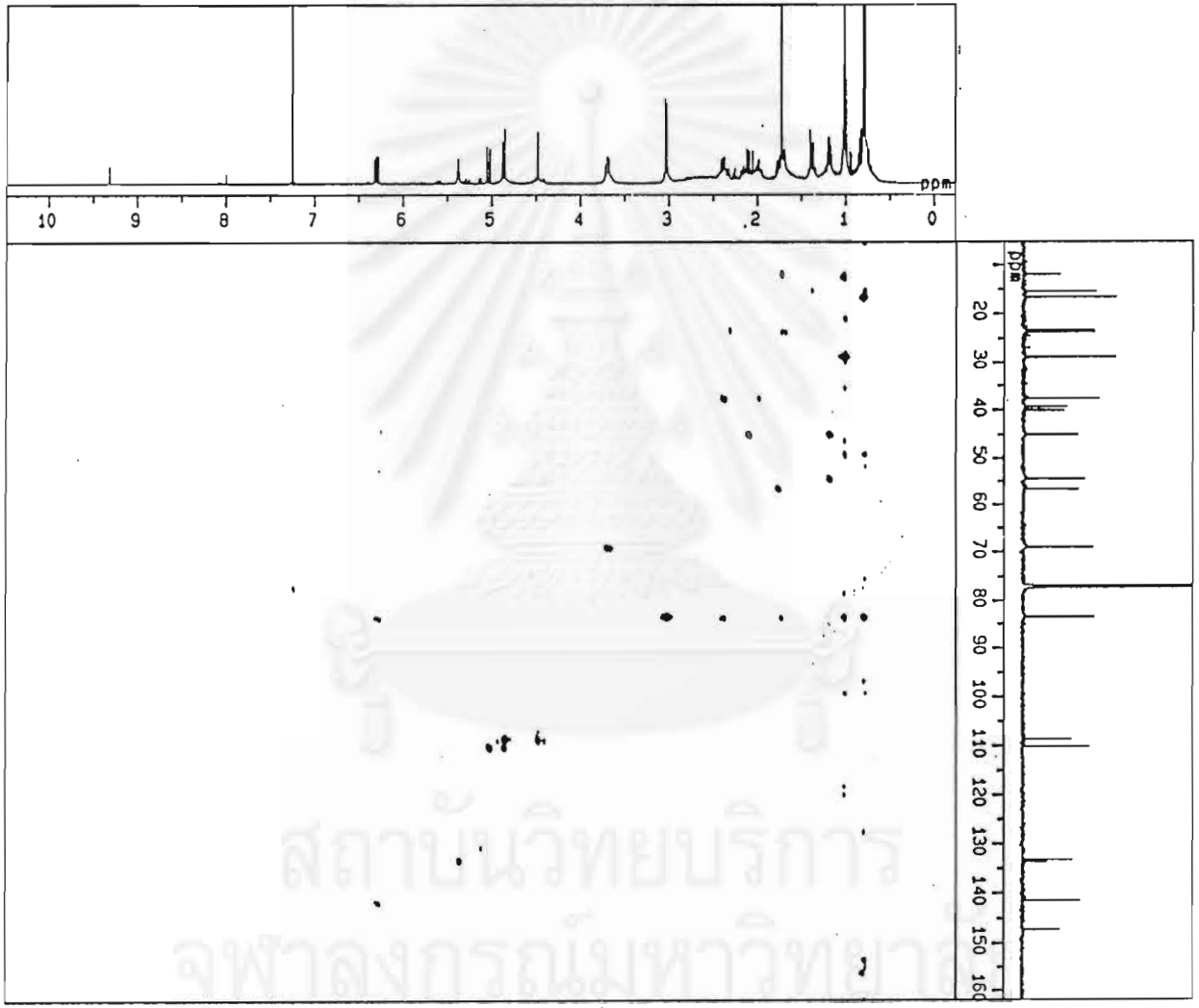


Figure 43 The HMQC (500 MHz) correlation spectrum of compound 3

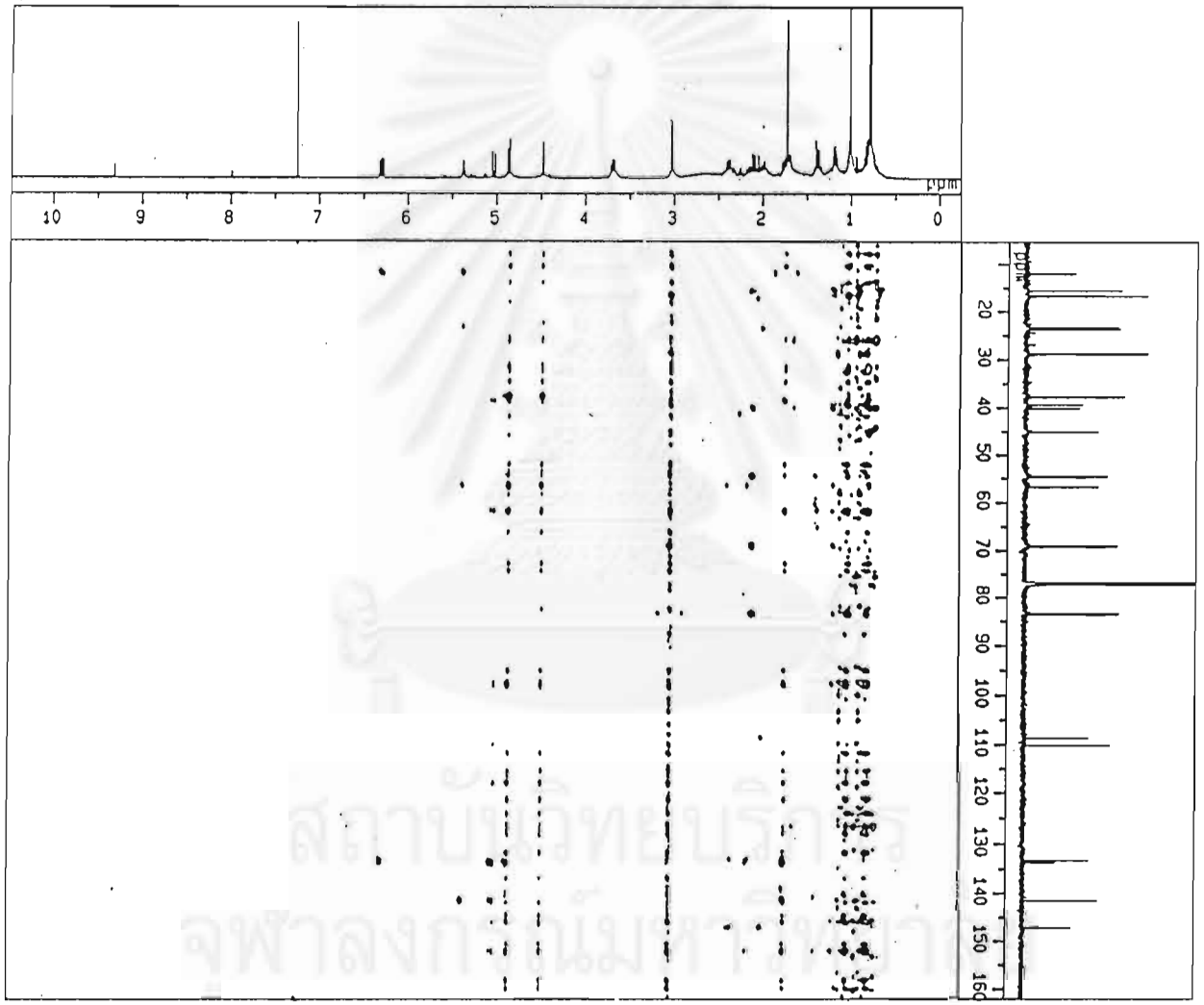


Figure 44 The HMBC (500 MHz) correlation spectrum of compound 3

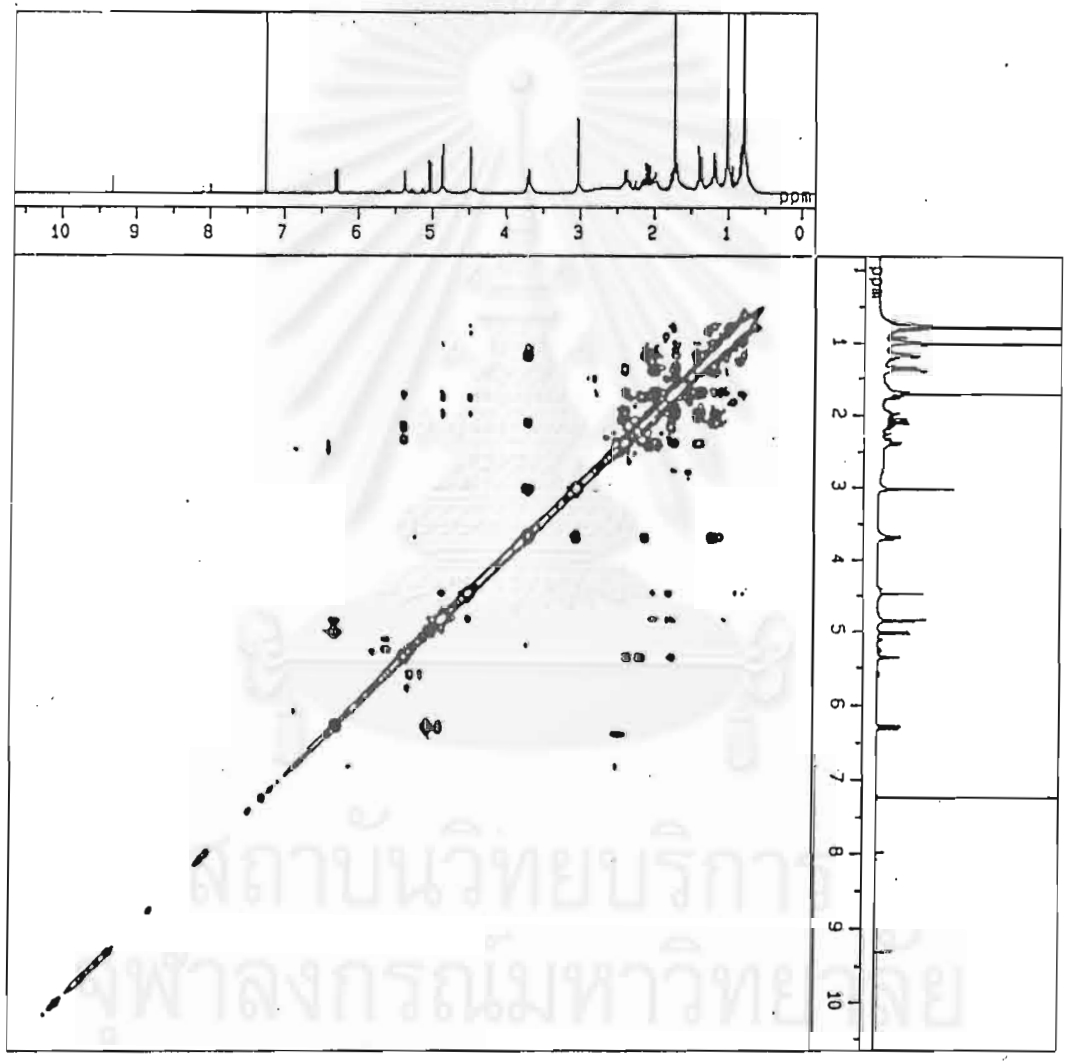


Figure 45 The COSY (500 MHz) spectrum of compound 3

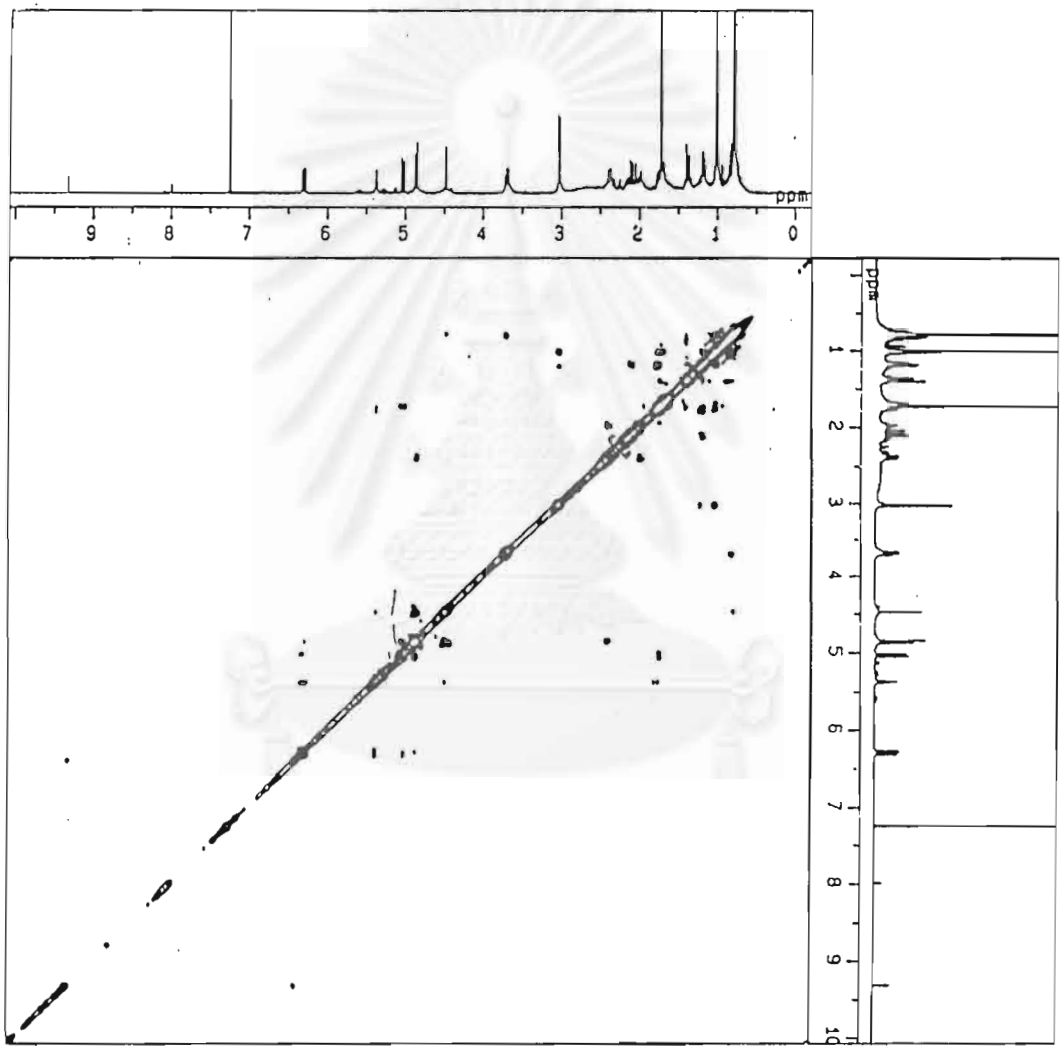


Figure 46 The NOESY (500 MHz) spectrum of compound 3

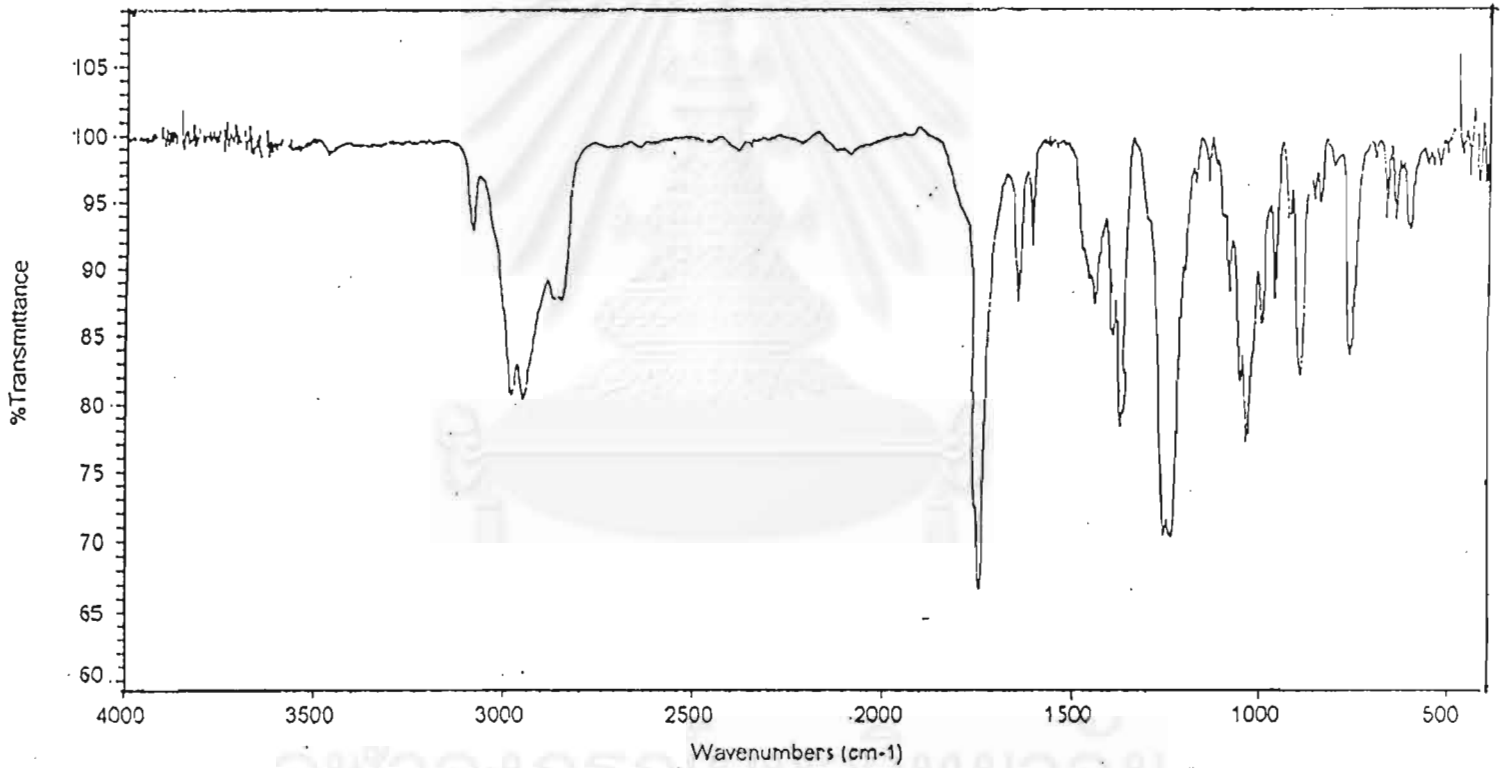


Figure 47 The IR spectrum of compound 4

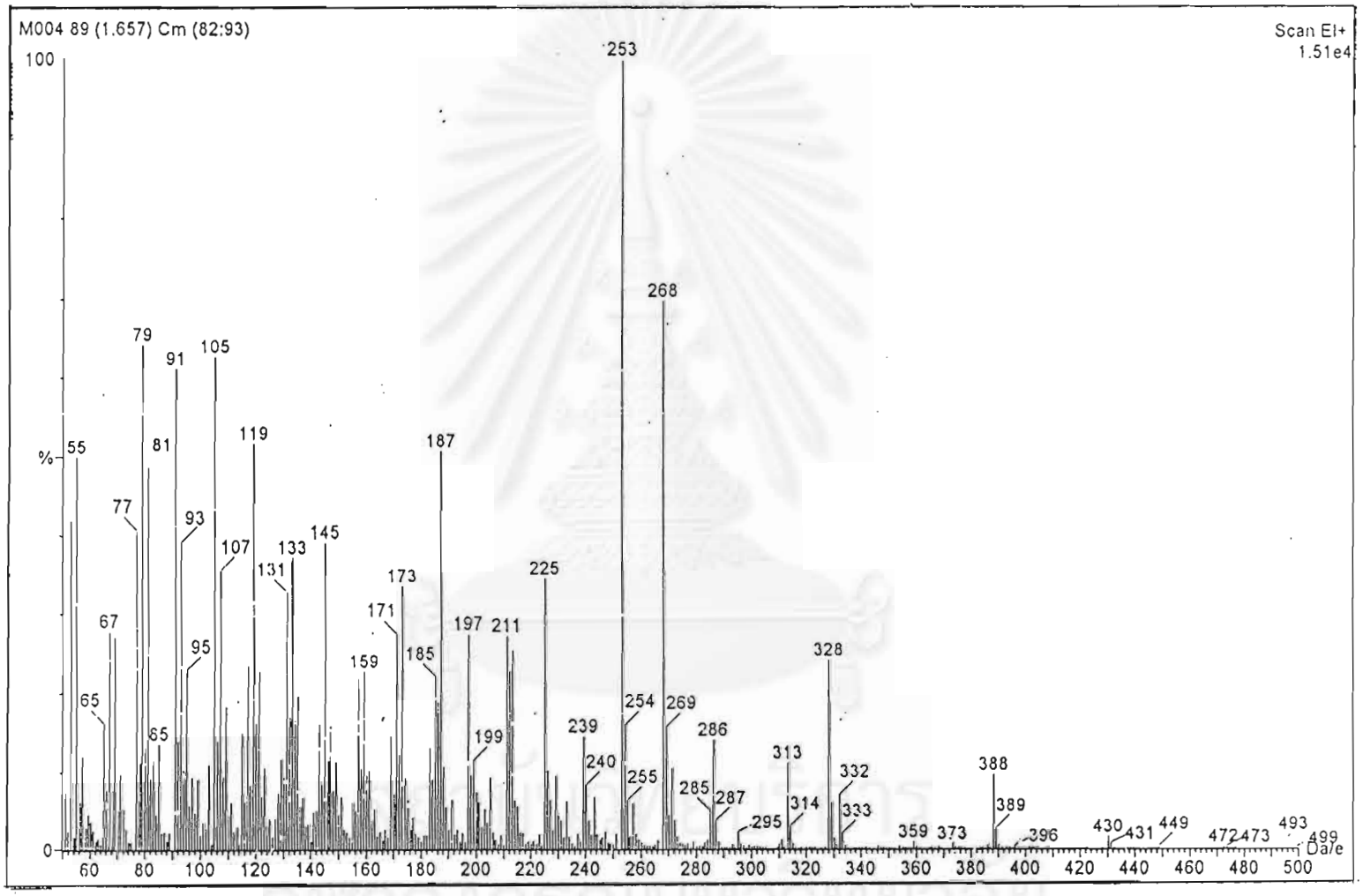


Figure 48 The EI mass spectrum of compound 4

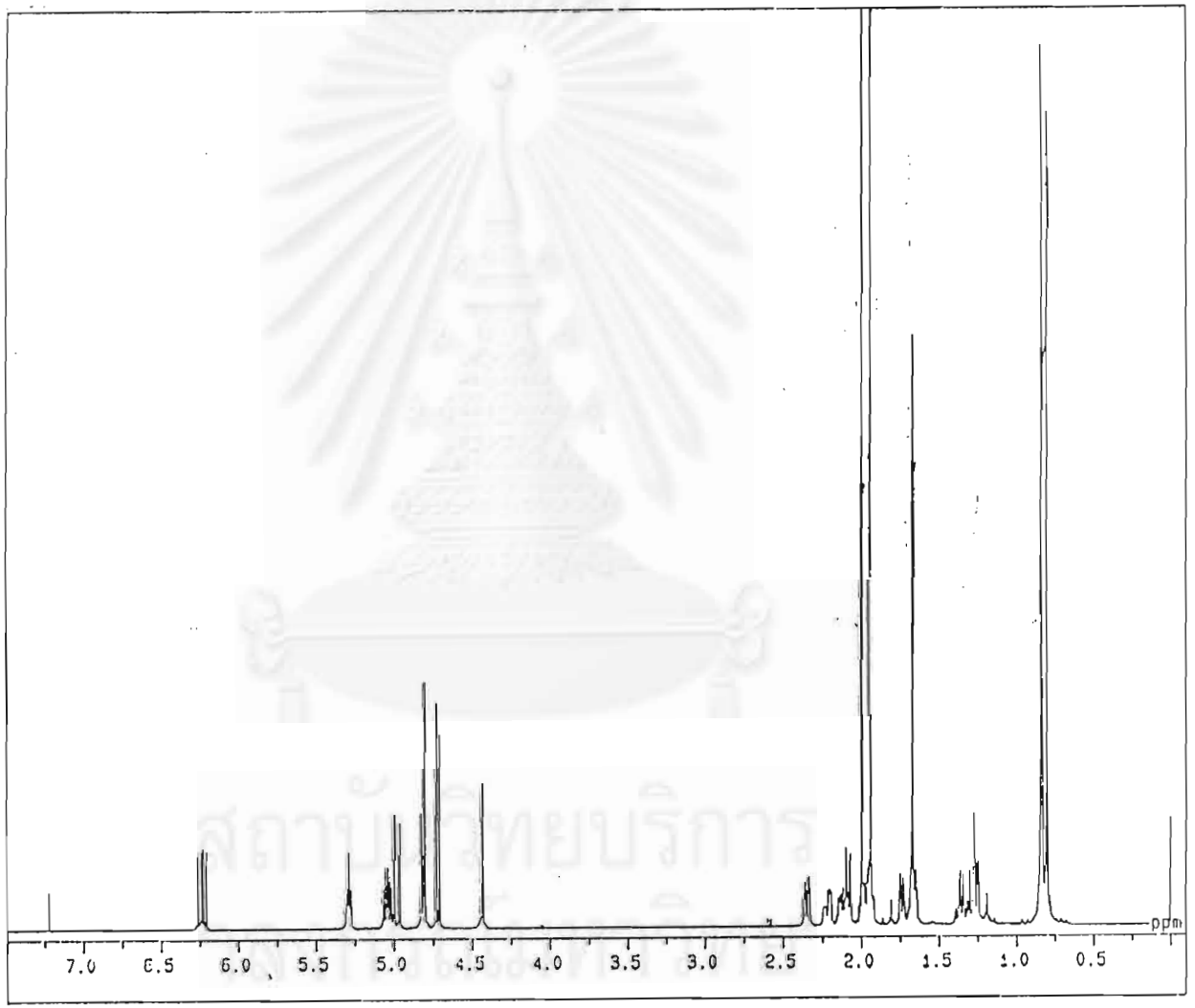


Figure 49 The ^{1}H NMR (500 MHz) spectrum of compound 4 (in $CDCl_3$)

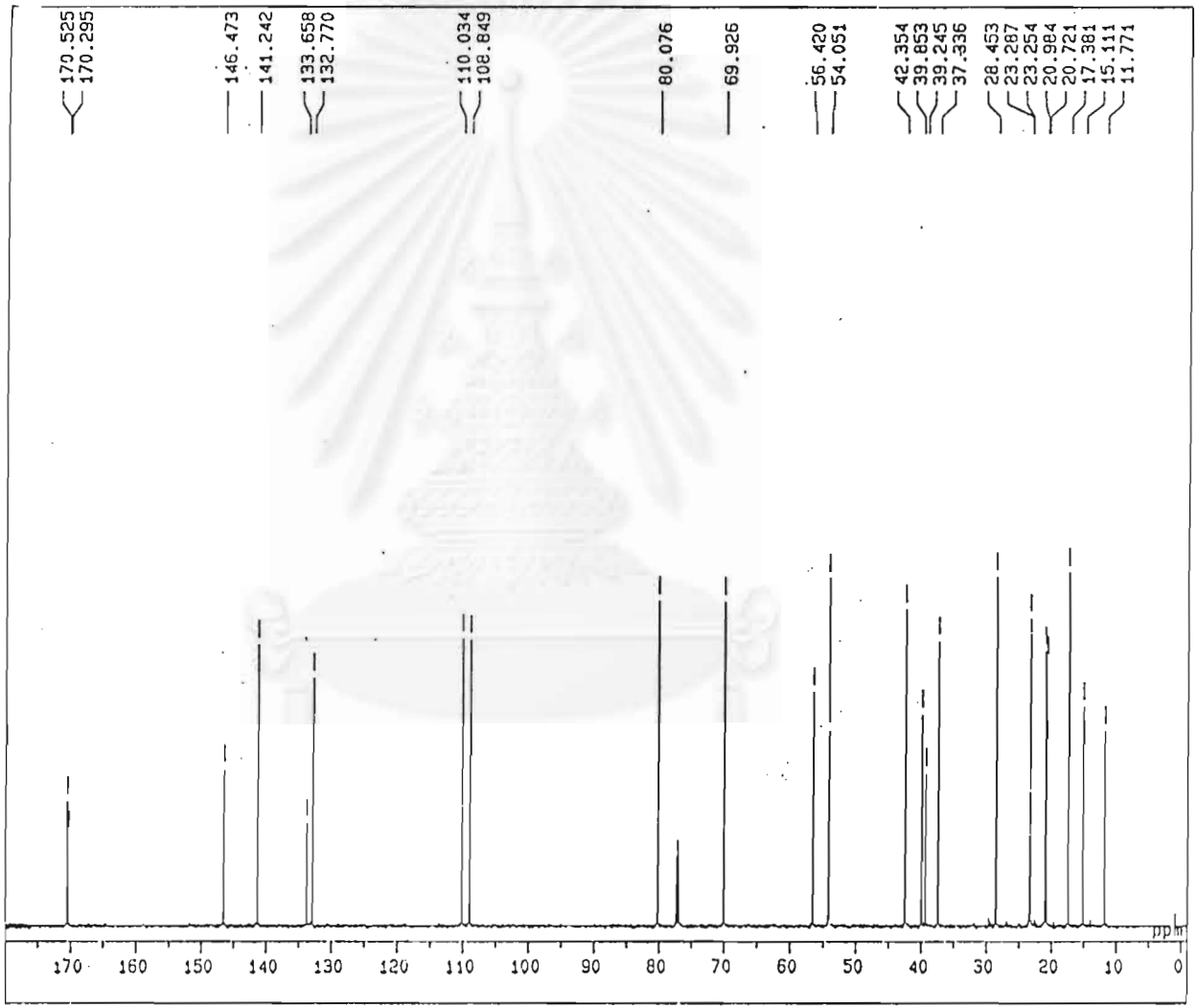


Figure 50 The ^{13}C NMR (125 MHz) spectrum of compound 4 (in $CDCl_3$)

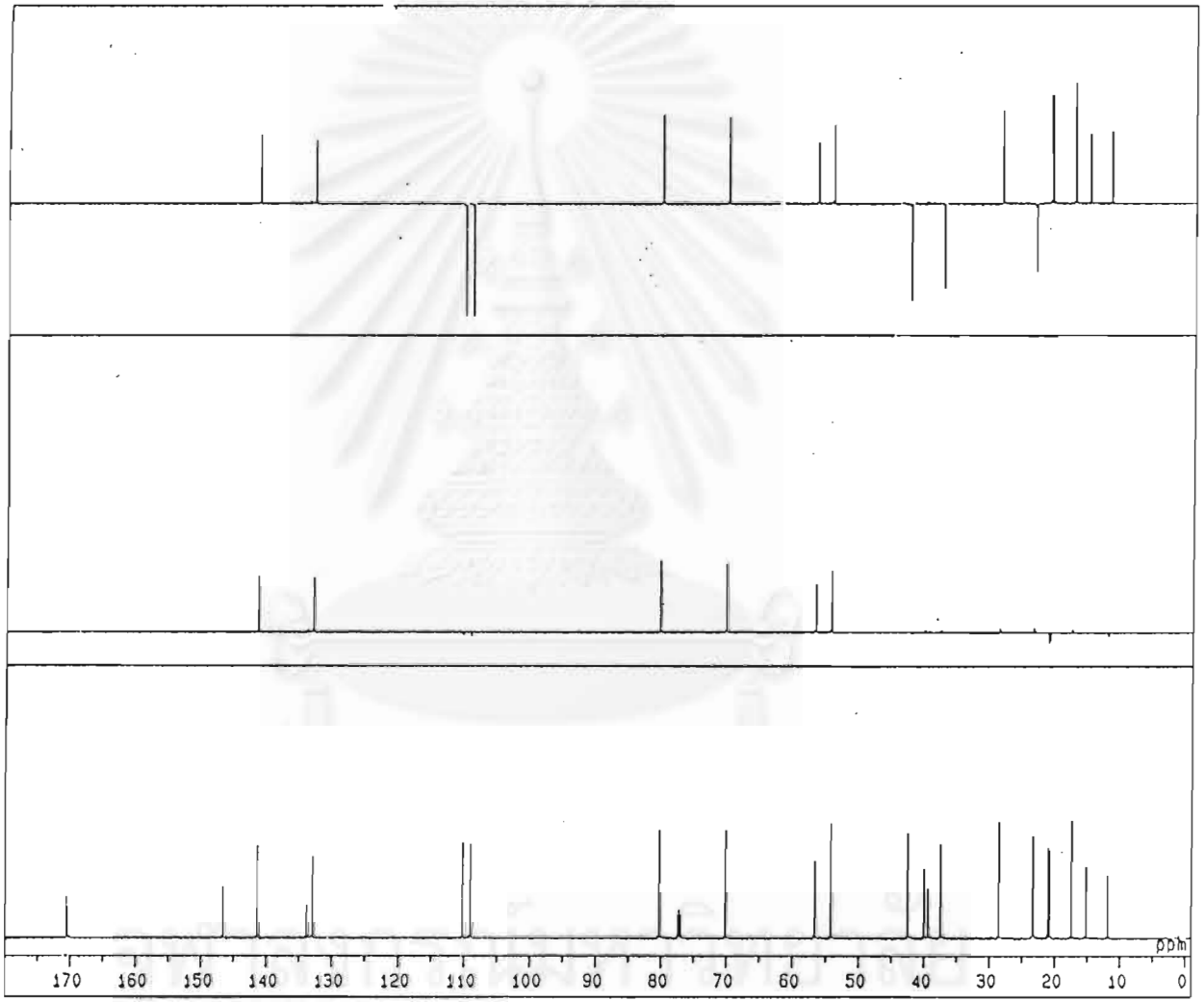


Figure 51 The DEPT (125 MHz) spectrum of compound 4 (in $CDCl_3$)

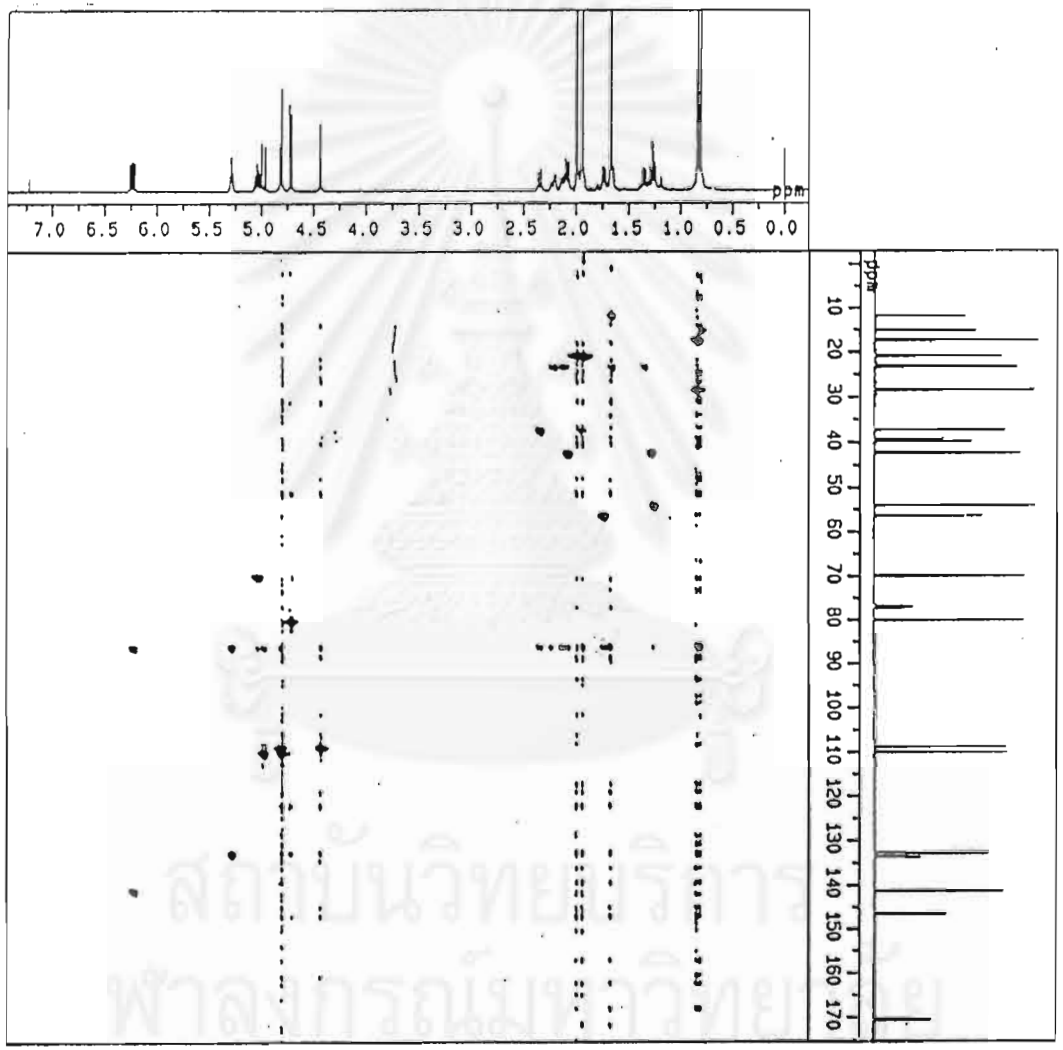


Figure 52 The HMQC (500 MHz) correlation spectrum of compound 4

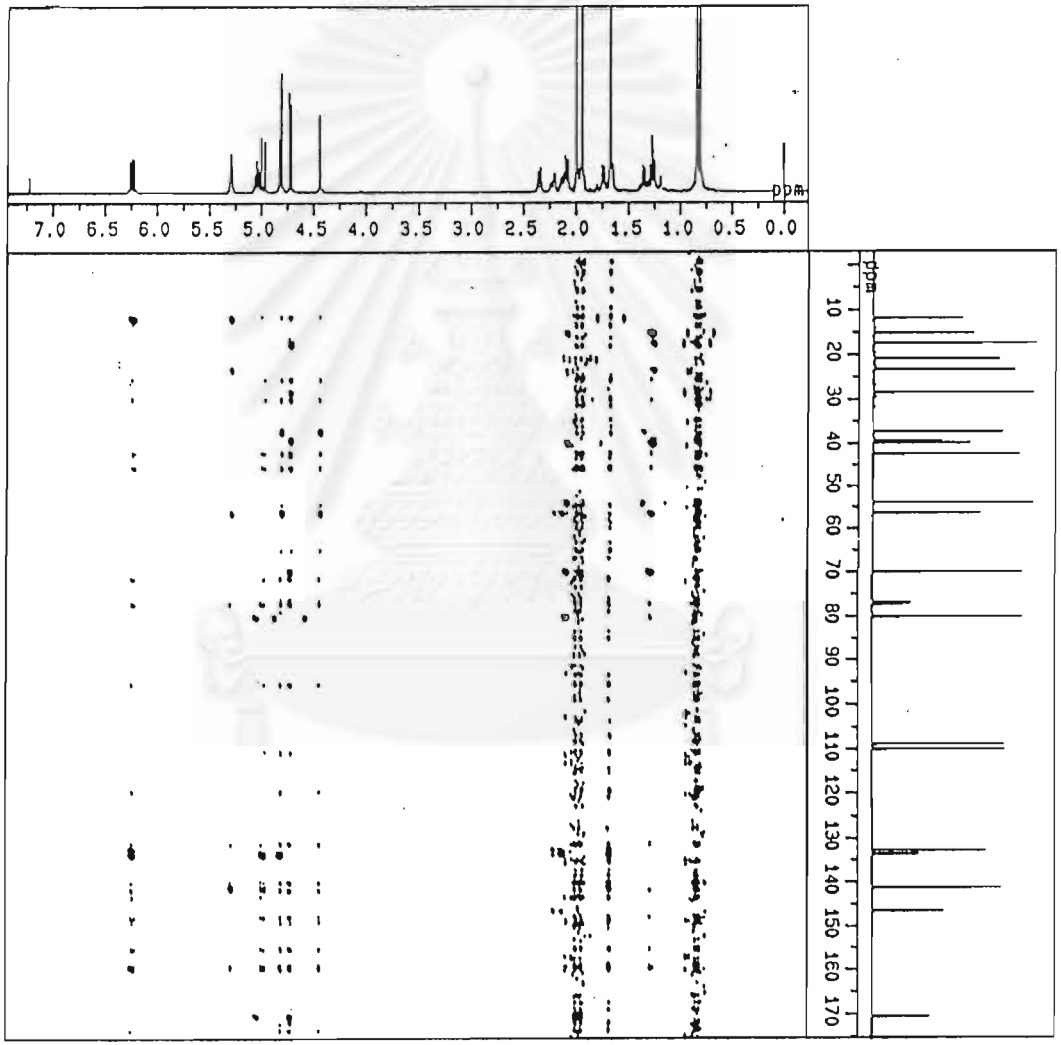


Figure 53 The HMBC (500 MHz) correlation spectrum of compound 4

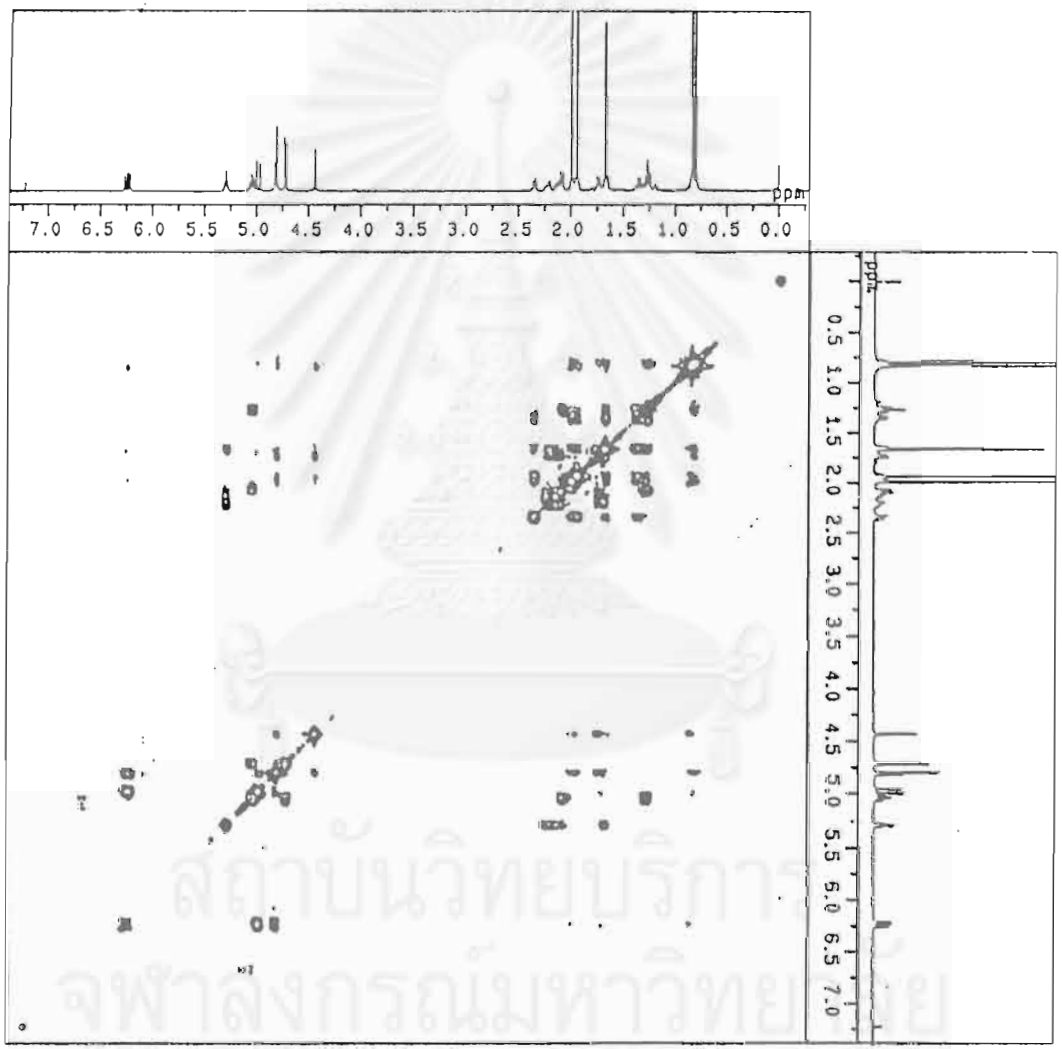


Figure 54 The COSY (500 MHz) spectrum of compound 4

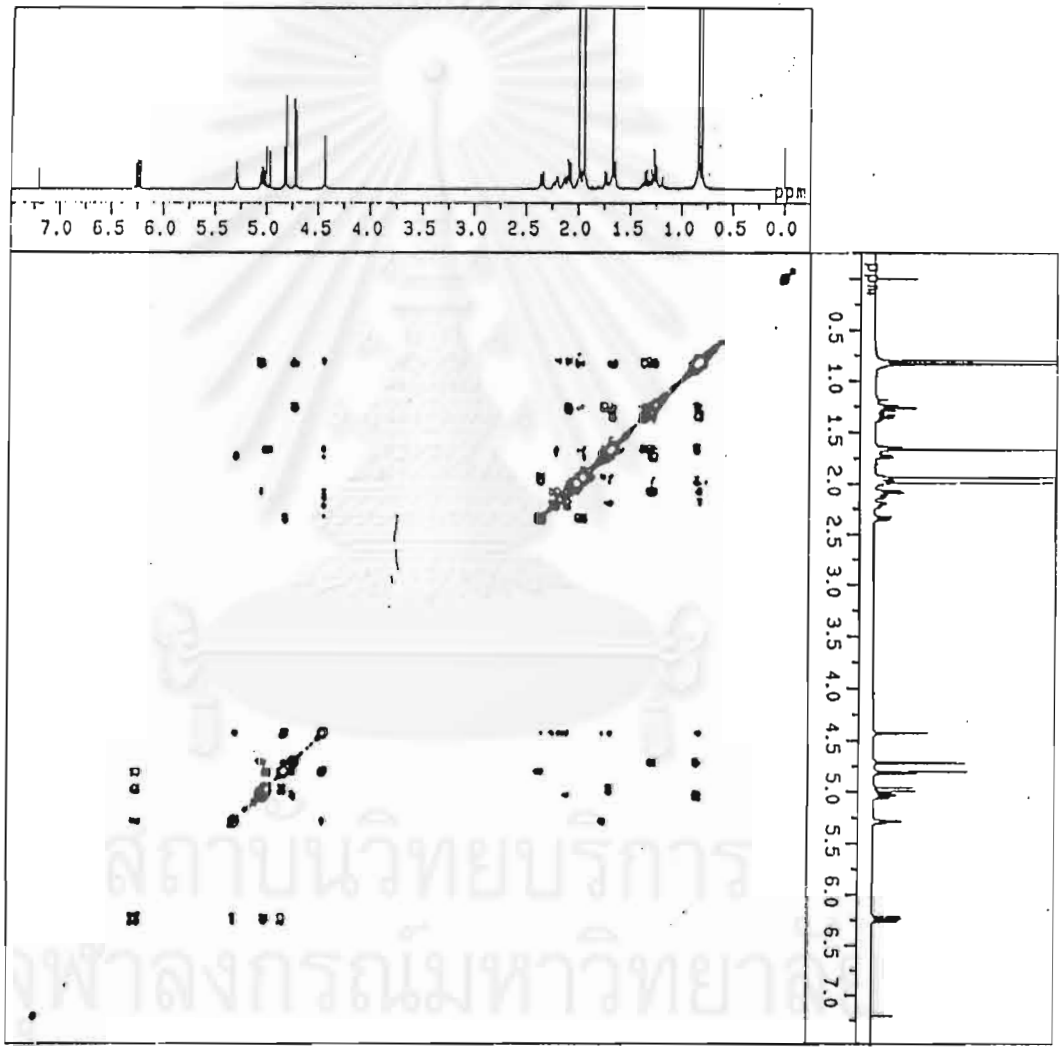


Figure 55 The NOESY (500 MHz) spectrum of compound 4

APPENDIX B

Mannscript submitted to *Phytochemistry*

Cytotoxic labdane diterpenoids from *Croton oblongifolius*

Sophon Roengsumran[a,*], Amorn Petsom[a,c], Narupat Kuptiyanuwat[a], Tirayut Vilaivan[a], Nattaya Ngamrojnavanich[a], Chaiyo Chaichantipyuth[b], Songchan Puthong[c]

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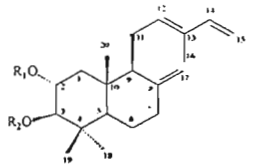

	R_1	R_2
1	Ac	H
2	H	Ac
3	H	H
4	Ac	Ac

Three new labdane diterpenoids were isolated from stem bark of *Croton oblongifolius*. The structure of these compounds were established by spectroscopic data and they were tested for cytotoxicity against various human tumor cell lines.

Cytotoxic labdane diterpenoids from *Croton oblongifolius*

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Abstract

Three new labdane diterpenoids, 2-acetoxy-labda-8(17),12(*E*)-14-triene-3-ol, 3-acetoxy-labda-8(17),12(*E*)-14-triene-2-ol, labda-8(17),12(*E*),14-triene-2,3-diol were isolated from stem bark of *Croton oblongifolius*. The structure of these compounds were established by spectroscopic data and they were tested for cytotoxicity against various human tumor cell lines.

Keywords : Croton oblongifolius; Euphorbiaceae; Labdane; Diterpenoid; Cytotoxic

1. Introduction

Our continuing investigation of *Croton oblongifolius* Roxb. (Euphorbiaceae) available in various part of Thailand have yielded several new diterpenoids. For example, crotocembraneic acid, neocrotocembraneic acid, neocrotocembranal (Roengsumran *et al.*, 1998, 1999a) are obtained from *C. oblongifolius* specimen from Petchaboon Province in central part of Thailand. Moreover, four new labdane diterpenoids have been isolated from specimen from Prachuab Kirikhan Province in the southern part of Thailnd (Roengsumran *et al.*, 1999b). In this paper we describe the isolation and characterization as well as cytotoxic activity against human tumor cell lines of three new labdane diterpenoids from *C. oblongifolius* specimen from Loei Province in the northern part of Thailand.

2. Results and discussion

The crude hexane extract of the stem bark of *C. oblongifolius* was separated by chromatography on a silica gel column using a hexane-ethyl acetate gradient system to obtain three new labdane diterpenoids **1-3**. Treatment of **3** with acetic anhydride in pyridine gave **4**.

The molecular formula of compound **1** was assigned as $C_{22}H_{34}O_3$ based on elemental analysis, ^1H and ^{13}C NMR (Table 1 and 2) and EIMS [M^+] (*m/z* 346). Its mass spectrum exhibited a peak at *m/z* 328 corresponding to [M^+-H_2O]. The ion at *m/z* 286 was formed by loss of acetic acid. A broad absorption band at 3463 cm^{-1} in the IR spectrum comfirmed the presence of the hydroxy group. The IR streching at 1722 cm^{-1} (C=O) and 1253 cm^{-1} (C-O) indicate the presence of ester moiety in addition to the C=C stretching at 1639 cm^{-1}. The ^{13}C NMR spectrum and DEPT experiments revealed the presence of 22 nonequivalent carbons, of which 15 are sp^3 [5 methyl, 4 methylene, 4 methine and 2 quaternary carbons] and six sp^2 [2 methylene, 2 methine and 2 quaternary carbons] hybridized (carbons), together with a carbonyl carbon of ester. The six vinylic (or sp^2) carbons were consistent with the presence of three double bonds in the molecule. The molecular formula, $C_{22}H_{34}O_3$, of compound **1** defined a degree of unsaturation of six, therefore compound **1** must consist of two rings in addition to the three double bonds and one carbonyl group.

The ^1H and ^{13}C NMR suggested that compound **1** possesses an acetate group (δ_H 2.10 and δ_C 21.4 for $\underline{C}H_3CO$), consistent with the EIMS data. The $-CH=C(CH_3)-CH=CH_2$

group gave a typical ^{1}H NMR spectrum for a labda-12(*E*),14-diene skeleton (δ 4.89, H-15, d, *J*=11.0; 5.05, H-15, d, *J*=17.4; 5.35, H-12, dd, *J*=6.4, 6.4; 6.31, H-14, dd, *J*=11.0 and 17.4; and 1.74, H_3-16, d, *J*=0.9) (Roengsumran *et al.*, 1998; Noma *et al.*, 1982; Bohlmann & Czersin 1979). The exomethylene group showed ^{1}H and ^{13}C NMR signals at δ_H 4.49 (H-17, br.d, *J*=1.5), 4.87 (H-17, br.d, *J*=1.5), and δ_C 108.8 (C-17, t). The ^{1}H NMR spectrum also showed three additional singlets due to three methyl groups at δ_H 0.85 (H_3-20), 0.87 (H_3-19) and 1.06 (H_3-18). Therefore, the OH and acetoxy moieties must be confined to the decalin ring system. A well-defined double doublets at δ_H 3.23 (H-3, *J*=5.2,10.0) was shown to be on C-3 carbon (δ_C 80.5) by HMQC experiment and it was shown to be correlated to a quaternary carbon at δ_C 39.9(C-4), two methyl carbons (δ_C 16.5, C-19 and 28.7, C-18) and an oxygen bearing methine carbon at δ_C 73.2 (C-2) by HMBC experiment. Thus the two oxygen bearing methine carbons must be adjacent to one another. The well-defined doublet of double doublets at δ_H 4.96 (H-2, *J*=4.3, 10.0 and 11.7) was shown to connect to δ_C 73.2 (C-2) by HMQC and correlated to δ_C 42.3 (C-1), 80.5 (C-3) and 171.6 (C=O) by HMBC experiment. Therefore, the acetoxy group must be on C-2 carbon. A large coupling constant of 10.0 Hz between H-2 and H-3 indicated that both of them were in axial orientation. Thus the OH and acetoxy moieties were in equatorial orientation. The unequivocal assignment of compound 1 was established by the information from HMQC, HMBC, COSY and NOESY experiments as shown in Table 1-3 and Figure 2. The spectral data of 2,3-dioxygenated decalin ring system with 8(17) exomethylene of compound 1 was also in agreement with those of methyl 2β, 3β-dihydroxy-labda-8(17),13*Z*-diene-15-oate isolated from *Nolana rostrata* (Garbarino *et al.*, 1986). Thus compound 1 was assigned as 2-acetoxy-labda-8 (17),12(*E*),14-triene-3-ol.

Compound 2 was isolated as a white solid with molecular formula $C_{22}H_{34}O_3$. ^{1}H and ^{13}C NMR spectra (Table 1 and 3) of compound 2 were similar to those of compound **1**. The HMBC experiment revealed the correlation of methine proton at δ_H 4.55 (H-3, d, *J*=10.1) to a quaternary carbon at δ_C 39.4 (C-4), an oxygen bearing methine carbon at δ_C 67.8 (C-2) and a C=O carbon at δ_C 172.4. Therefore, the acetoxy group located on the C-3 carbon. The two protons on C-2 and C-3 showed axial relationship as suggested by the large coupling constant of 10.1 Hz as in compound **1**. Thus compound 2 was assigned as 3-acetoxy-labda-8 (17),12(*E*),14-triene-2-ol.

Compound 3 was shown to have a formula of $C_{20}H_{32}O_2$ by elemental analysis. ^1H and ^{13}C NMR data for 3 were similar to those of compound 1 and 2 except the absence of acetyl group. Therefore, compound 3 was labda-8(17),12(*E*),14-triene-2,3-diol.

Compound 4 was obtained from peracetylation of compound 3. It gave expected spectroscopic properties (IR, MS, ^1H and ^{13}C NMR).

Compounds (**1-4**) were tested for their cytotoxicity against human tumor cell lines and compound **3** showed moderate activities against human gastric carcinoma (KATO-3, 2.2 μg/mL), colon carcinoma (SW 620, 2.7 μg/mL), breast carcinoma (BT474, 4.6 μg/mL), hepatocarcinoma (HEP-G2, 3.7 μg/mL) and lung carcinoma (CHAGO, 3.3 μg/mL). Compound **4** was inactive against all cell lines (>10 μg/mL). Compound **1** was active against gastric (5.7 μg/mL) and colon carcinoma (7.1μg/mL) while compound 2 was active against gastric (3.3 μg/mL) and breast carcinoma (5.9 μg/mL). Therefore, the presence of acetyl group did not enhance cytotoxicity of compound **3**. Perhaps, monoacetylation (compound 1 and 2) and diacetylation (compound 4) of compound 3 could render their ability to form hydrogen bond with certain receptor on tumor cells and made them inactive.

3. Experimental

Mps uncorr.: on Fisher Johns melting point apparatus. Optical rotation: on a Perkin-Elmer 341 polarimeter. UV spectra: on a Hewlett Packard 8452A. IR spectra: on a Nicolet Impact 410 Spectrophotometer. Low-resolution MS: 70 eV. on a Fisons Instruments Trio 2000 mass spectrometer. ^1H and ^{13}C NMR, HMQC and HMBC experiments were carried out on a JEOL JNM-A500 spectrometer. Microanalyses were performed at Chulalongkorn Research Equipment Centre on a Perkin Elmer PE 2400 Series II.

3.1 Plant material

The stem bark of *C. oblongifolius* was collected from Ampur Wang Saphung, Loei Province, Thailand, in May 1998. Botanical identification was achieved through comparison with a voucher specimen No. BKF 084729 in the herbarium collection of the Royal Forest Department of Thailand.

3.2 Extraction and isolation

The powdered, sun-dried stem bark of *C. oblongifolius* (6.5 kg) was extracted with hexane. The hexane extract was filtered, and evaporated *in vacuo* to obtain a greenish yellow oil (300 g). The hexane extract (100 g) was fractionated by silica gel CC and eluted with hexane-EtOAc to obtain compound **1**(120 mg, 0.005%), **2** (60 mg, 0.003%) and **3** (8.94 g, 0.41%) from 5%, 10% and 30% EtOAc in hexane, respectively.

3.3 Biological evaluation

Bioassay of cytotoxic activity against human tumor cell culture *in vitro* was performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetic method (Carmichael et at., 1987; Twentyman & Luscombe 1987).

3.4 2-acetoxy-labda-8(17),12(E),14-triene-3-ol (1)

White solid, mp. 102-103° (Found C, 76.24 ; H, 9.91 $C_{22}H_{34}O_3$ requires: C 76.26; H, 9.89%); $[\alpha]_D^{20}$ +50.17 ($CHCl_3$; c 1.0); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244sh (4.45); IR ν_{max}^{KBr} cm^{-1}: 3463 (OH), 2974, 2940, 2863, 1722 (C=O), 1639 (C=C), 1441, 1373, 1252 (C-O), 1030; ^1H and ^{13}C NMR: Table 1 and 3. EIMS *m/z* (rel. int.): 346[M^+] (24), 331[M^+-CH_3](6), 328[M^+-H_2O](4), 317(7), 304(8), 290(15), 286[M^+-CH_3COOH](75), 272(16), 271(72), 268 (63), 255(25), 253(100), 243(49), 230(28), 229(43), 225(34), 213(33), 191(30), 187(46), 185(32), 173(37), 171(27), 147(31), 145(41), 133(32), 131(40), 119(40), 105(57), 91(70), 79(42), 77(66).

3.5 3-acetoxy-labda-8(17),12(E),14-triene-2-ol (2)

White solid, mp. 99-101° (Found C, 76.25 ; H, 9.89 $C_{22}H_{34}O_3$ requires: C 76.26; H, 9.89%); $[\alpha]_D^{20}$ +9.46 ($CHCl_3$; c 1.0); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244sh (4.53); IR ν_{max}^{KBr} cm^{-1}: 3443 (OH), 2940, 2848, 1722 (C=O), 1644, 1605, 1460, 1373, 1248(C-O), 1030; ^1H and ^{13}C NMR: Table 1 and 3. EIMS *m/z* (rel. int.):346[M^+] (9), 328[M^+-H_2O](4), 313(1), 286[M^+-CH_3COOH](19), 271(45), 268(32), 255(22), 253(78), 243(39), 229(44), 213(32), 203(27), 187(52), 173(67), 159(56), 147(68), 145(60), 135(73), 133(87), 121(80), 119(95), 107(82), 105 (100), 95(53), 93(68), 91(70), 81(67), 79(89).

3.6 labda-8(17),12(E),14-triene-2,3-diol (3)

White solid, mp 69-70° (Found C, 78.87 ; H, 10.59 $C_{20}H_{32}O_2$ requires: C 78.90; H, 10.59%); $[\alpha]_D^{20}$ -6.96 ($CHCl_3$; c 1.0); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244.5sh (4.14); IR ν_{max}^{KBr} cm^{-1}:3376 (OH), 2969, 2945, 2853, 1644, 1610, 1446, 1388, 1214(C-O), 1059; ^1H and ^{13}C NMR: Table 2 and 3. EIMS *m/z* (rel. int.):304[M^+] (68), 289[M^+-CH_3](53), 286[M^+-CH_3COOH](22), 271(75), 253(33), 248(62), 233(45), 215(27), 201(26), 187(78), 173(42), 159(46), 147(61), 145(70), 133(76), 119(76), 105(94), 91(100), 79(84).

3.7 2,3-diacetoxy labda-8(17),12(E),14-triene (4)

Compound **4** was prepared by acetylation of **3** (100 mg, 0.33 mmol) with acetic anhydride (80 mg, 0.78 mmol) in pyridine. The reaction product was purified by silica gel CC eluting with 2% EtOAc in hexane to give **4** as viscous oil (85 mg, 66%); (Found C, 74.19; H, 9.34 $C_{24}H_{36}O_4$ requires: C 74.19; H, 9.34%); $[\alpha]_D^{20}$ +24.52° ($CHCl_3$; c 1.0); UV $\lambda_{max}^{CHCl_3}$ nm (log ε):232sh (4.39); IR ν_{max}^{neat} cm^{-1}: 2979, 2955, 2850, 1732(C=O), 1645, 1613, 1440, 1384,1255(C-O), 1040; ^{13}C NMR: table 2 and 3. EIMS *m/z* (rel. int.): 388[M^+] (11), 373(1), 328[M^+-CH_3COOH](25), 313(13), 286(17), 268[M^+-$2CH_3COOH$] (70), 253 (100), 239(15), 225(33), 211(38), 197(38), 187(52), 173(33), 159(23), 145(39), 133(38), 119(51), 105(62), 91(60), 79(63).

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References

Bohlmann, F. & Czerson, H. (1979). *Phytochemistry*, *18*, 115.

Carmichael, J., DeGraff, W.G., Gazdar, A. F., Minna, J.D. & Mitchell, B. (1987). *Cancer Res.*, *47*, 936.

Garbarino, J., Chamy, M.C. & Gambaro, V. (1986). *Phytochemistry*, *25*, 2833.

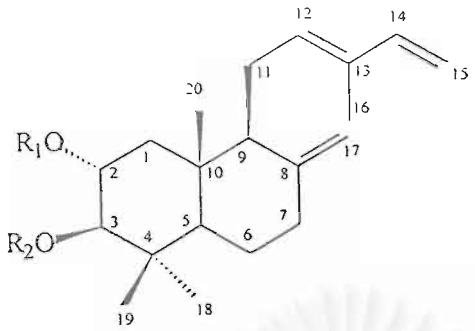
Noma, M., Suzuki, F., Gamou, K. & Kawashima, N. (1982). *Phytochemistry*, *21*, 395.

Roengsumran, S., Achayindee, S., Petsom, A., Pudhom, K., Singtothong, P.,Surachetapan, C. & Vilaivan, T. (1998). *J. Nat. Prod.*, *61*, 652.

Roengsumran, S., Singtothong, P., Pudhom, K., Ngamrochanavanich, N., Petsom, A., & Chaichantipyuth, C. (1999a). *J. Nat. Prod.*, *62*, 1163.

Roengsumran, S., Petsom, A., Sommit, D. & Vilaivan, T. (1999b). *Phytochemistry*, *50*, 449.

Twentyman, P.R. & Luscombe, M. (1987). *Br. J. Cancer*, *56*, 279.



	R_1	R_2
1	Ac	H
2	H	Ac
3	H	H
4	Ac	Ac

Figure 1

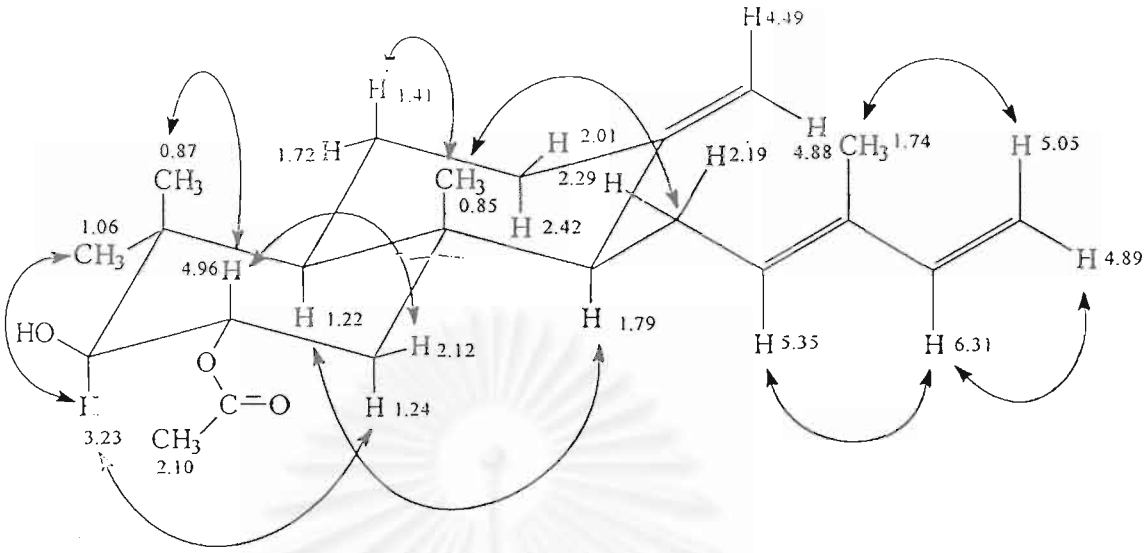


Figure 2.

Table 1

^{1}H and ^{13}C NMR data (δ in ppm. *J* in Hz) for compound **1** and **2** in $CDCl_3$

	Compound 1		Compound 2	
Position	δ_H	HMBC (H to C)	δ_H	HMBC (H to C)
1	1.25 dd (*J*=11.7,11.7)	C-2,C-3,C-9,C-10,C-20	1.29 dd (*J*=12.1,12.1)	C-2,C-3,C-9,C-10,C-20
	2.12 dd (*J*=4.3,11.7)	C-2,C-3,C-5,C-10,C-20	2.21 dd (*J*=4.3,12.1)	C-2,C-3,C-5,C-10,C-20
2	4.96 ddd (*J*=4.3,10.0,11.7)	C-1,C-3,C-18,C=O	3.81 ddd (*J*=4.3,10.1,11.6)	C-1,C-3,
3	3.23 dd (*J*=5.2,10.0)	C-2,C-4,C-18,C-19	4.55 d (*J*=10.1)	C-1,C-2,C-4,C-18,C-19,C=O
5	1.22 dd (*J*=2.7,12.6)	C-4,C-6,C-9,C-10,C-18,C-19,C-20	1.29 dd (*J*=2.4,12.8)	C-4,C-6,C-10,C-20
6	1.41 dddd (*J*=4.3,12.6,12.6,12.6)	C-5,C-7,C-8,C-10	1.41 dddd (*J*=4.3,12.8,12.8,12.8)	C-5,C-7
	1.74 m	C-4,C-5	1.72 dddd (*J*=2.6,5.2,5.2,12.8)	C-5
7	2.00 dd (*J*=5.2,13.1)	C-6,C-8	2.02 ddd (*J*=4.9,13.1,13.1)	C-6,C-8,C-17
	2.42 ddd (*J*=2.4,4.3,13.1)	C-5,C-6,C-8,C-9	2.41 ddd (*J*=2.4,4.3,13.1)	C-5,C-8,C-9,C-17
9	1.79 br.d (*J*=11.6)	C-8,C-10,C-11,C-20	1.80 br.d (*J*=10.4)	C-8,C-10
11	2.19 dd (*J*=6.4,10.6)	C-8,C-9	2.19 m	C-8,C-9,C-12,C-13
	2.29 br.dd (*J*=4.3,15.0)	C-8,C-9,C-12,C-13	2.36 m	C-8,C-9,C-12,C-13
12	5.35 dd (*J*=6.4,6.4)	C-9,C-11,C-13,C-14,C-16	5.40 dd (*J*=6.4,6.4)	C-9,C-11,C-14,C-16
14	6.31 dd (*J*=11.0,17.4)	C-12,C-13,C-16	6.32 dd (*J*=11.0,17.4)	C-12,C-13,C-15,C-16
15	4.89 d (*J*=11.0)	C-13	4.89 d (*J*=11.0)	C-13
	5.05 d (*J*=17.4)	C-13,C-14	5.05 d (*J*=17.4)	C-13,C-14
16	1.74 d (*J*=1.0)	C-11,C-12,C-13,C-14,C-15	1.75 d (*J*=0.9)	C-12,C-13,C-14
17	4.49 br.d (*J*=1.5)	C-7,C-9	4.51 br.d (*J*=1.5)	C-7,C-8,C-9
	4.87 br.d (*J*=1.5)	C-7,C-9	4.87 br.d (*J*=1.5)	C-7,C-9
18	1.06 s	C-3,C-4,C-5,C-19	0.90 s	C-3,C-4,C-5,C-19
19	0.87 s	C-3,C-4,C-5,C-18	0.87 s	C-3,C-4,C-5,C-18
20	0.85 s	C-1,C-5,C-9,C-10	0.80 s	C-1,C-5,C-9,C-10
$\underline{CH_3}CO$	2.10 s	C=O	2.15 s	C=O

Table 2

^{1}H and ^{13}C NMR data (δ in ppm. *J* in Hz) for compound **3** and **4** in $CDCl_3$

	Compound 3		Compound 4	
Position	δ_H	HMBC (H to C)	δ_H	HMBC (H to C)
1	1.18 dd (*J*=11.7,12.5)	C-2,C-3,C-9,C-10,C-20	1.27 dd (*J*=11.9,12.3)	C-2,C-3,C-9,C-10,C-20
	2.10 dd (*J*=4.6,12.5)	C-2,C-3,C-5,C-10,C-20	2.08 dd (*J*=4.6,12.3)	C-2,C-3,C-5,C-10,C-20
2	3.69 ddd (*J*=4.3,9.6,11.7)	-	5.04 ddd (*J*=4.6,10.5,11.9)	C-3,C=O
3	3.02 d (*J*=9.6)	C-1,C-2,C-4,C-18,C-19	4.71 d (*J*=10.5)	C-2,C-4,C-18,C-19,C=O
5	1.19 dd (*J*=2.7,12.5)	C-10,C-20	1.25 dd (*J*=2.7,12.8)	C-4,C-6,C-10,C-20
6	1.40 dddd (*J*=4.3,12.5,12.5,12.5)	-	1.35 dddd (*J*=4.3,12.8,12.8,12.8)	C-5,C-7
	1.71 m	C-5	1.65 m	C-7
7	1.99 m	C-6,C-8,C-17	1.96 m	C-8
	2.39 ddd (*J*=2.4,4.0,12.8)	C-5,C-8,C-9	2.34 ddd (*J*=2.1,4.0,13.1)	-
9	1.76 br.d (*J*=10.7)	-	1.73 br.d (*J*=9.8)	C-8,C-10
11	2.17 dd (*J*=6.7,11.0)	C-9,C-12	2.12 m	C-8,C-9,C-12,C-13
	2.34 br.dd (*J*=5.5,11.0)	C-8,C-12	2.20 m	C-8,C-9,C-12,C-13
12	5.38 dd (*J*=6.1,6.1)	C-9,C-11,C-14,C-16	5.28 dd (*J*=6.4,6.4)	C-9,C-11,C-14,C-16
14	6.29 dd (*J*=11.0,17.4)	C-12,C-13,C-16	6.25 dd (*J*=10.7,17.4)	C-12,C-13,C-16
15	4.86 d (*J*=11.0)	C-13	4.81 d (*J*=10.7)	C-13
	5.02 d (*J*=17.4)	C-13,C-14	4.98 d (*J*=17.4)	C-13,C-14
16	1.72 d (*J*=0.9)	C-12,C-13,C-14	1.66 d (*J*=0.9)	C-12,C-13,C-14
17	4.47 br.d (*J*=1.2)	C-7,C-9	4.43 br.d (*J*=1.2)	C-7,C-9
	4.85 br.d (*J*=1.2)	C-7,C-9	4.80 m	C-7,C-9
18	1.01 s	C-3,C-4,C-5,C-19	0.83 s	C-3,C-4,C-5,C-19
19	0.80 s	C-3,C-4,C-5,C-18	0.84 s	C-3,C-4,C-5,C-18
20	0.78 s	C-1,C-5,C-9,C-10	0.80 s	C-1,C-5,C-9,C-10
$\underline{CH_3}CO$	-	-	1.93 s	C=O
$\underline{CH_3}CO$	-	-	1.99 s	C=O

Table 3
^{13}C NMR spectral data of compound **1-4** (125 MHz, $CDCl_3$, ppm)

Position	1	2	3	4
1	42.3	46.3	45.0	42.4
2	73.2	67.8	69.1	70.0
3	80.5	84.5	83.5	80.2
4	39.9	39.4	39.3	39.3
5	54.4	54.4	54.5	54.1
6	23.5	23.5	23.6	23.4
7	37.6	37.6	37.7	37.4
8	146.3	146.9	147.2	146.6
9	56.6	56.6	56.7	56.5
10	40.2	40.1	40.1	39.9
11	23.4	23.3	23.3	23.3
12	133.1	133.0	133.2	132.8
13	133.7	133.8	133.7	133.8
14	141.4	141.5	141.5	141.3
15	110.1	110.2	110.1	110.1
16	11.9	11.9	11.9	11.9
17	108.8	108.8	108.6	108.9
18	28.7	28.7	28.8	28.5
19	16.5	17.5	16.6	17.5
20	15.2	15.4	15.4	15.2
C=O	171.6	172.4	-	170.3
C=O	-	-	-	170.5
$\underline{CH_3}$-C=O	21.4	21.2	-	21.0
$\underline{CH_3}$-C=O	-	-	-	20.8

Table 4.
Cytotoxicity data of compounds 1-4[a]

	Cell lines				
Compound	KATO-3	SW620	BT 474	HEP-62	CHAGO
1	5.7	7.1	>10	>10	>10
2	3.3	>10	5.9	>10	>10
3	2.2	2.7	4.6	3.7	3.3
4	>10	>10	>10	>10	>10

[a]Results are expressed as IC_{50} values (μg/mL)

[b]KATO-3 : Human gastric carcinoma ATCC No. HTB 103
SW620 : Human colon carcinoma
BT474 : Human breast carcinoma ATCC No. HTB 20
HEP-G2 : Human hepatocarcinoma ATCC No. HB 8065
CHAGO : Human lung carcinoma

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