ผลของหมู่แทนที่ของ 4-ไฮดรอกซีคูมารินต่อฤทธิ์ทางชีวภาพ

นางสาว รวยรัชต์ วรกิจธำรงค์ชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2543
ISBN 974-347-274-6

EFFECT OF SUBSTITUENTS OF 4-HYDROXYCOUMARINS ON THEIR BIOLOGICAL ACTIVITIES

Miss. Rouirush Worakijthamrongchai

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2000

ISBN 974-347-274-6

Thesis Title	Effect of Substituents of 4-Hydroxycoumarins on Their Biological Activities
By	Miss Rouirush Worakijthamrongchai
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Warinthorn Chavasiri, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

.....Dean of Faculty of Science
(Associate Professor Wanchai Phothiphichitr, Ph.D.)

Thesis committee:

.....Chairman
(Professor Udom Kokpol, Ph.D.)

.....Thesis Advisor
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.....Member
(Professor Padet Sidisunthorn, Ph.D.)

.....Member
(Associate Professor Pipat Karntiang, Ph.D.)

.....Member
(Associate Professor Kingkaew Wattanasirmkit, Ph.D.)

รวยรัชต์ วรกิจธำรงค์ชัย : ผลของหมู่แทนที่ของ 4-ไฮดรอกซีคูมารินต่อฤทธิ์ทางชีวภาพ (EFFECT OF SUBSTITUENTS OF 4-HYDROXYCOUMARINS ON THEIR BIOLOGICAL ACTIVITIES) อ. ที่ปรึกษา ผศ.ดร. วรินทร ชวศิริ; 115 หน้า; ISBN 974-347-274-6.

ได้สังเคราะห์สารในกลุ่ม 4-ไฮดรอกซีคูมารินสิบสองสาร โดยอาศัยปฏิกิริยาคอนเดนเซชันระหว่างไดเอทิลคาร์บอเนต กับสารกลุ่ม 2-ไฮดรอกซีแอซีโทฟีโนน และสารในกลุ่มไดคูมารอลสี่สิบเอ็ดสาร โดยการทำปฏิกิริยาแทนที่ด้วยนิวคลีโอไฟล์ ระหว่าง 4-ไฮดรอกซีคูมาริน กับอัลดีไฮด์ที่สนใจ และได้นำสารเหล่านี้มาศึกษาฤทธิ์ทางชีวภาพสี่ชนิด สำหรับฤทธิ์ความเป็นพิษต่อไรสีน้ำตาล พบว่าไดคูมารอลที่มีหมู่เทอเซอรีบิวทิลสองหมู่ (**D37**) แสดงฤทธิ์ทางชีวภาพสูง โดยทั่วไปสารกลุ่ม 4-ไฮดรอกซีคูมารินแสดงฤทธิ์ทางชีวภาพที่สูงกว่าสารกลุ่มไดคูมารอล สารในกลุ่ม 4-ไฮดรอกซีคูมารินที่มีหมู่เมทอกซีแทนที่ที่ตำแหน่ง C-5 (**3**) แสดงแนวโน้มฤทธิ์ยับยั้งการเจริญเติบโตของต้นอ่อนของไมยราบยักษ์ที่ดี ในส่วนของฤทธิ์ความเป็นพิษเฉียบพลันต่อปลานิล พบว่า ไดคูมารอลสองชนิด ได้แก่ 3,3′-(methylene) *bis*-4-hydroxycoumarin and 3,3′-(4-nitrobenzylidene) *bis*-4-hydroxycoumarin แสดงฤทธิ์ทางชีวภาพสูง สำหรับฤทธิ์ต้านแบคทีเรีย พบว่า ไดคูมารอลที่มีหมู่เมทอกซี (**D25**) แสดงฤทธิ์ทางชีวภาพสูง จากผลที่ได้รับสนับสนุนความคิดที่ว่า ชนิดและตำแหน่งของหมู่แทนที่มีผลกระทบต่อฤทธิ์ทางชีวภาพ

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....รวยรัชต์ วรกิจธำรงค์ชัย...

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

ปีการศึกษา.....2543.....

4172404023 : MAJOR CHEMISTRY

KEY WORD : DICOUMAROL / 4-HYDROXYCOUMARIN / BIOLOGICAL ACTIVITY

ROUIRUSH WORAKIJTHAMRONGCHAI : EFFECT OF SUBSTITUENTS OF 4-HYDROXYCOUMARINS ON THEIR BIOLOGICAL ACTIVITIES. THESIS ADVISOR : ASSISTANT PROFESSOR WARINTHORN CHAVASIRI, Ph.D. 115 pp. ISBN 974-347-274-6.

Twelve 4-hydroxycoumarins derived from the condensation of diethyl carbonate and 2-hydroxyacetophenones and forty-one dicoumarols achieved from nucleophilic substitution reaction between 4-hydroxycoumarins and interesting aldehydes. These compounds were subjected to four bioassays. For brine shrimp lethality test, dicoumarol with two tertiary butyl substituents (**D37**) displayed high activities. In general 4-hydroxycoumarins were found to exhibit higher activity than dicoumarols. Among 4-hydroxycoumarins, a methoxy group at C-5 (**3**) showed a good tendency in weed growth inhibition against *Mimosa pigra* Linn. In the case of acute toxicity test against *Oreochromis niloticus*, high activities were observed in two dicoumarols, namely, 3,3′-(methylene) *bis*-4-hydroxycoumarin and 3,3′-(4-nitrobenzylidene) *bis*-4-hydroxycoumarin. For antibacterial activity, dicoumarol with a methoxy group (**D25**) revealed high activity. These obtained results manifestly endorsed the conceptual ideas that the type and position of substituents affected the biological activity.

Department.....Chemistry.....Student's signature Rouirush Worakijthamrongchai

Field of study .....Chemistry.....Advisor's signature Warinthorn Chavasiri

Academic year.....2000.....

ACKNOWLEDGEMENT

The author would like to express her sincere gratitude to her advisor Assistant Professor Dr. Warinthorn Chavasiri, for his kindly assistance, valuable suggestions and encouraging throughout the course of this research. Sincere thanks are also extended to Professor Dr. Udom Kokpol, Professor Dr. Padet Sidisunthorn, Associate Professor Dr. Pipat Karntiang and Associate Professor Kingkaew Wattanasirmkit, her thesis committee, for their comments and suggestions.

Gratitude is also expressed to the staff of the Natural Products Research Unit, Department of Chemistry, Chulalongkorn University for their helpful discussion and support. In addition, the author greatly appreciated the Faculty of Science, Chulalongkorn University for granting her a research assistantship during 1998-2000 and partial financial support to this research work.

The auther also thanks to her bioassay supports: Associate Professor Kingkaew Wattanasirmkit (Department of Biology, Chulalongkorn University), for providing and giving an advice in Brine Shrimp Lethality Test and Acute Toxicity Test, Mrs. Siriporn Stonsaovapak (Institute of Food Research and Product Development, Kasetsart University) for the performance of antibacterial bioassay and Dr. Siriporn Zungsontiporn (Division Botany of Weed Science Sub-division, Botany and Weed Science Division, Department of Agriculture, Ministry of Agriculture and Cooperatives) for kindly testing of antigrowth activities.

Special thanks are given to S. Wattanasereekul, W. Sirisuksukon and P. Boonsong for providing some chemicals.

A deep gratitude is acknowledged to her parents and family members for their love, understanding, encouragement and support throughout the entire course of study.

CONTENTS

List of Figures

Figs **Page**

Figs **Page**

List of Tables

Tables **Page**

List of Schemes

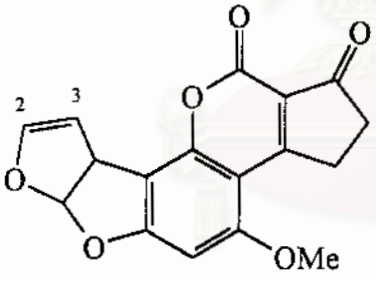
Schemes **Page**

List of Abbreviations

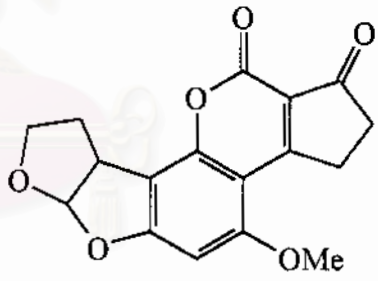
br	broad	m.p.	melting point
°C	degree Celsius	MS	mass spectroscopy
cm^{-1}	unit of wavenumber	*m/z*	mass per charge
Cpd	compound	NMR	nuclear magnetic resonance
d	doublet (NMR)	ppm	part per million
dd	doublet of doublet (NMR)	quart	quartet (NMR)
dt	doublet of triplet (NMR)	quint	quintet (NMR)
DMSO	dimethylsulfoxide	rel. int.	relative intensity
g	gram (s)	R_f	retardation factor
h	hextet (NMR)	s	strong (IR)
Hz	hertz	s	singlet (NMR)
IR	infrared	t	triplet (NMR)
J	coupling constant	w	weak (IR)
lit	literature	wt	weight
m	multiplet (NMR)	δ	chemical shift
m	medium (IR)	%	percent
mL	milliliter (s)		

CHAPTER I
INTRODUCTION

A search for new therapeutic compounds for drug development has been still extensively continued. The new compounds may be of discovered through direct isolation from natural sources or by chemical synthesis. Moreover, many attempts to increase the potential of compounds have been accomplished by studying the relationship between structure and activity. The results from these investigations firmly indicated that part(s) of structure such as functional groups or substituents effect biological activity of compounds. For example, Wogen *et al.* concluded that the furofuran moiety and the 2,3 double bond were both essential for toxic and carcinogenic potency. Aflatoxin B_1 **(I)** produced 78% numbers of tumors at 20 ppb in young fish whereas aflatoxin B_2 **(II)** produced only 5% numbers of tumors at the same concentration.[1]



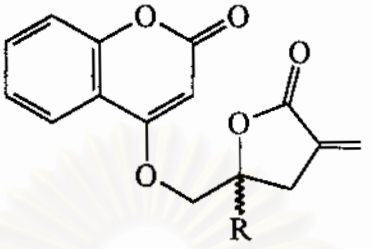
(I)



(II)

Another example could be visualized from a search for the inhibitors of platelet aggregation. Certain coumarin derivatives were synthesized and evaluated for antiplatelet activity against arachidonic acid (AA)- and platelet-activating factor (PAF)- induced aggregation in washed rabbit platelet. Compound **(III)**, with methyl substituent at C-2 of the lactone moiety, was shown to be less active than compound **(IV)**, with phenyl substituent at C-2. Compounds **(V-VIII)**, possessing a substituted phenyl group at C-2,

were found to have broad antiplatelet activities in which both AA- and PAF- induced aggregation were strongly inhibited.[2]



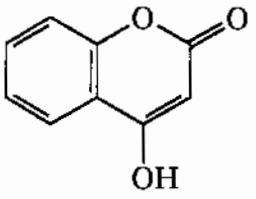
Compound	R	IC_{50} (μM)	
		AA	PAF
(III)	CH_3	191.21	278.67
(IV)	Ph	8.21	103.67
(V)	4-F-C_6H_4	14.14	14.58
(VI)	4-Cl-C_6H_4	8.99	22.92
(VII)	4-CH_3-C_6H_4	10.02	10.02
(VIII)	4-OCH_3-C_6H_4	12.08	12.77

Thus, the synthesis of natural lead compounds and their derivatives are necessary for evaluating the relationship between structure and activity.

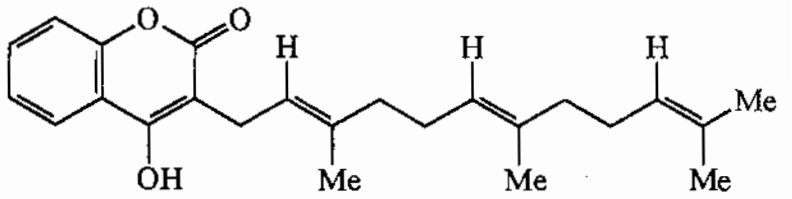
1.1 Literature Reviews

1.1.1 4-Hydroxycoumarins

4-Hydroxycoumarin **(IX)** is a small group in part of coumarins substituted in a pyrone ring. This compound occurs widely in plants and has been shown to possess biological activity in many different organisms. For instance, ferulenol **(X)**, 3-farnesyl-4-hydroxycoumarin derivative, was isolated from the root sap of *Ferula communis* which exhibited haemorrhagic activity.[3]



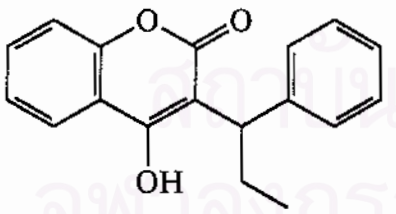
(IX)



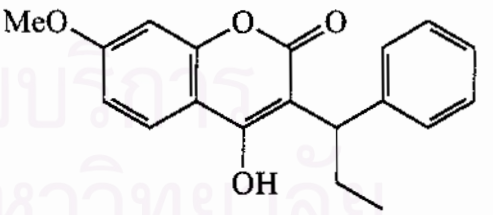
(X)

Because of attractive bioactivities of 4-hydroxycoumarins, there are numerous methods developed for the synthesis of these compounds and applied to evaluate the structure-activity relationship. For example, early methods to synthesize these compounds are treating methyl acetylsalicylate with sodium metal at high temperature to obtain the ring closure *via* an intramolecular Claisen condensation.[4] Another reported methodology was the condensation of phenols with malonic acids in the presence of zinc chloride and phosphorus oxychloride.[5]

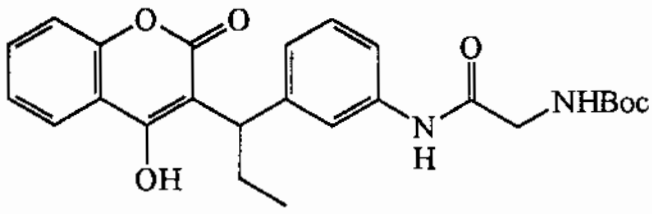
In addition, from a broad screening program to discover nonpeptide HIV protease inhibitors, phenprocoumon (**XI**, Ki = 1 μM) was previously identified as a lead template. Compound **(XII)**, containing a methoxy group, and compound **(XIII)**, with amino acid, were prepared and shown to have improved inhibitory activity over the reference compound **(XI)** (Ki = 0.56 and 0.16 μM, respectively).[6]



(XI)

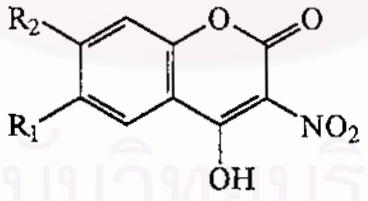


(XII)

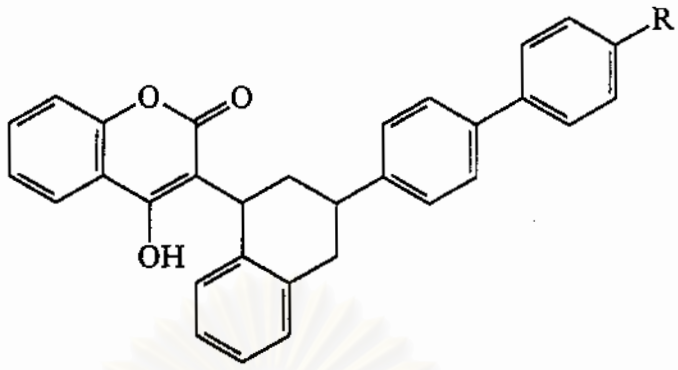


(XIII)

In 1975, the rat passive cutaneous anaphylaxis test was used to evaluate a series of 4-hydroxy-3-nitrocoumarin **(XIV)** as potential antiallergic compounds. This report indicated that alkyl substituents at positions C-6 and C-7 in 4-hydroxy-3-nitrocoumarins (Compounds **XV**, **XVI** and **XVII**) showed good activity.[7] Subsequently, the improvement of the existing syntheses of the anticoagulants diphenacoum **(XVIII)** and brodifacoum **(XIX)** was succeeded with a marked increase in overall yield. This method used commercially available starting materials and limited the number of steps to five and six.[8] More recent research, two simple and inexpensive methods for the preparation of 4-hydroxycoumarin from aspirin were described. The synthetic strategies involved the use of well-known ethyl acetoacetate synthesis and malonic ester synthesis.[9]



Compound	R_1	R_2	ED_{50} (mg/kg)
(XIV)	H	H	9.1
(XV)	CH_3	CH_3	1.0
(XVI)	C_2H_5	CH_3	1.3
(XVII)	C_2H_5	C_2H_5	0.4

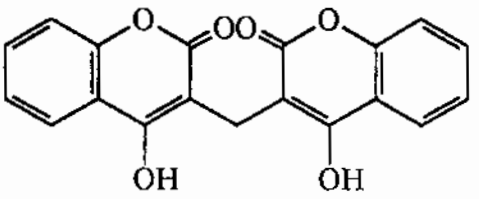


(XVIII) R = H

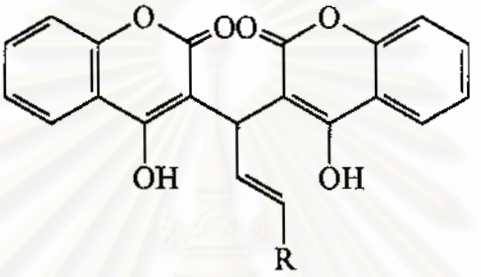
(XIX) R = Br

1.1.2 Dicoumarols

Dicoumarol **(XX)**, a biscoumarins class, was found in the aerial part and seed of *Hedysarum hedysaroides*. This compound is best known for its anticoagulant effect on blood.[1] Later, many studies have attempted to explore other activities of dicoumarols and their derivatives. A efficient report route is for example, the condensation of two equimolars of 4-hydroxycoumarin with several aliphatic and aromatic aldehydes.[10] In the similar methodology, another search reported that 4-hydroxycoumarin reacts with substituted salicylaldehydes, 2,6-dichloro or chloro-nitrobenzaldehydes to yield unstable 3,3′-benzylidene-bis-(4-hydroxycoumarin) intermediates which undergo ring closure with the hydroxyl group of the hydroycoumarin moieties, producing derivatives of 7-[4′-hydroxycoumarinyl-(3′)]benzopyrano[3,2-c]coumarins.[11] Another route particularly for the synthesis of dicoumarol was simply prepared by condensation of the 4-hydroxycoumarin with aqueous formaldehyde solution.[12] Moreover, dicoumarol derivatives (**XXI**, **XXII** and **XXIII**) were reported to obtain from treating substituted enals at room temperature with 4-hydroxycoumarin and ethylenediammonium diacetate in methanol as solvent.[13]



(XX)

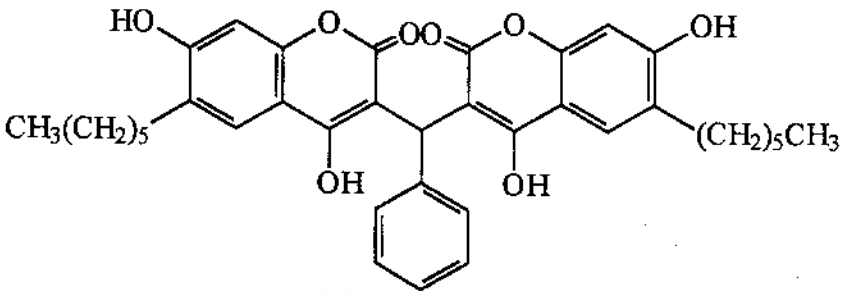


(XXI) R = 4-$NO_2C_6H_4$

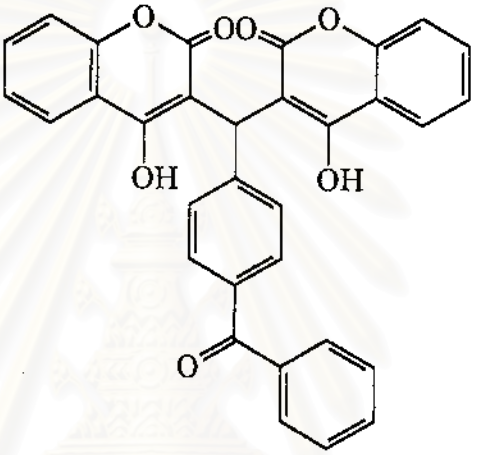
(XXII) R = Ph

(XXIII) R = PhCH=CH (*E*)

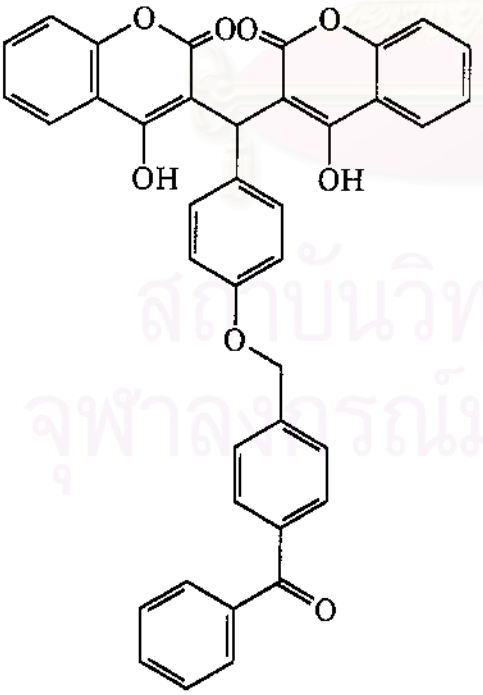
In 1976, adenosine-triphosphate phosphoribosyltransferase from *Escherichia coli* was inhibited by dicoumarol in competition with ATP (K_I was appoximately 60 μM). This compound diminished the yield of phosphoribosyladenosine triphosphate in the transferase reaction apparently by acting as parasite substrates.[14] The intensive investigation of anti-HIV drugs still proceeds, recent reports have shown that the best non-peptide HIV protease inhibitor was 3,3′-benzylidene-bis-(6-hexyl-4,7-dihydroxycoumarin) **(XXIV)**, a dimer of 4-hydroxycoumarin, with ID_{50} value of 0.32 μM.[15] Subsequently, three dimeric coumarin analogues (**XXV**, **XXVI** and **XXVII**) were synthesized, each containing the photoactivatable benzophenone moiety. These compounds exhibited low micromolar IC_{50} values against HIV-1 integrase mediated 3′-processing and strand transfer.[16]



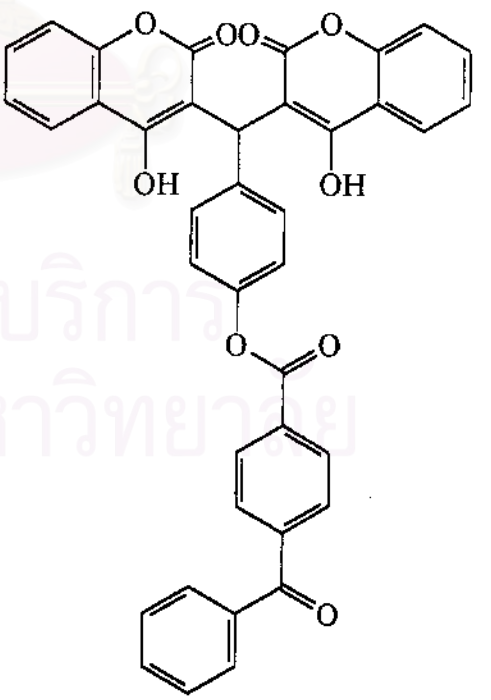
(XXIV)



(XXV)



(XXVI)



(XXVII)

In addition, some 4-hydroxycoumarins and dicoumarols were synthesized and evaluated the relationship between their structures and insect antifeedant against *Galleria mellonella* Linn. and weed growth inhibition against *Mimosa pigra* Linn. It was found that when hydroxy and methoxy substituents were present on a benzylidene ring of dicoumarols, those compounds displayed high activity.[17] Very recently, two types of dicoumarols and 3-alkyl-4-hydroycoumarins were prepared and subjected to three bioassays. In general, 3-alkyl-4-hydroycoumarins revealed higher brine shrimp cytotoxicity and antibacterial activity than their analogous dicoumarols. In the case of antiviral activity against HSV-1 and HSV-2 virus, it was observed that the activity of dicoumarols against HSV-1 was higher than HSV-2 but *vice versa* for 3-alkyl-4-hydroycoumarins.[18]

1.2 Aim of Research

This research is designed to develop 4-hydroxycoumarins and dicoumarols for utilizing in pharmaceutical and agricultural purposes. The study on the effects of structure is important for understanding the biological activities of compounds. Even though 4-hydroxycoumarins and dicoumarols were reported to possess broad spectrum of activities, the evaluation of relationship between structure and some activities of these compounds still goes on and needs for further examination. In summary, the purposes of this research are:

1. To synthesize 4-hydroxycoumarins and dicoumarols
2. To study effect of the substituents of 4-hydroxycoumarins and dicoumarols on their biological activities: brine shrimp cytotoxic lethality test, weed growth inhibition, static acute toxicity bioassay of Tilapia, and antibacterial activity

CHAPTER II
EXPERIMENTAL SECTION

2.1 Instruments and Equipment

All melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck Kieselgel 60 PF_{254}). Column chromatography was carried out on silica gel (Merck Kieselgel 60, 70-230 mesh). The FT-IR spectra were recorded on a Fourier-Transform Infrared Spectrophotometer model Impact 410: solid samples were incorporated to potassium bromide to form a pellet while liquid samples were dropped on a sodium chloride cell. The ^{1}H- and ^{13}C-NMR spectra were performed in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal reference on a Bruker model ACF 200 spectrometer which operated at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei and a Joel model JNM-A500 which operated at 500.00 MHz for ^{1}H and 125.00 MHz for ^{13}C nuclei. The chemical shifts were assigned by comparison with residue solvent protons. Mass spectra (70eV) were obtained from a Fission Instrument mass spectrometer Model VG TRIO 2000 in EI mode.

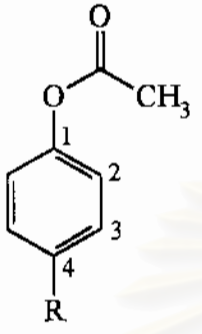
2.2 Chemicals

All solvents used in this research were purified prior to use by standard methodology except for those which were reagent grades. The reagents used for synthesizing the precursors, 4-hydroxycoumarins and dicoumarols were purchased from Fluka Chemical Company or otherwise stated and were used without further purification.

2.3 Synthesis

2.3.1 Synthesis of 4-Hydroxycoumarins and Starting Materials

Preparation of Phenyl acetates



Compound	R
2a	CH_3
6a	Cl
10a	Ph

General Procedure: Acetic anhydride was added to a solution of phenol in pyridine. The reaction mixture was refluxed for 2 hours. The mixture was poured into ice water and extracted with dichloromethane. The combined organic layer was then extracted with 2 N hydrochloric acid and 1 N sodium hydroxide, respectively and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to give the crude product.[19]

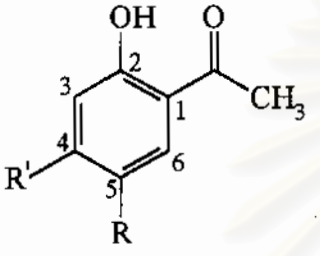
4-Methylphenyl acetate[20] **(2a):** Colorless liquid (85%), 86-87°C (4 mm Hg) (lit[20] b.p. 212-213°C), R_f 0.66 (chloroform). IR (neat, cm^{-1}): 3029, 2929, 1770, 1600, 1444, 1376, 1200 and 1012; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.24 (3H, s), 2.31 (3H, s), 6.94 (2H, d, J = 8.24 Hz) and 7.14 (2H, d, J = 8.54 Hz).

4-Chlorophenyl acetate[20] **(6a):** Colorless liquid (84%), 88-89°C (4 mm Hg) (lit[20] b.p. 226-228°C), R_f 0.51 (chloroform). IR (neat, cm^{-1}): 3107, 2925, 1778, 1595, 1487, 1379, 1196 and 1091; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.29 (3H, s), 7.03 (2H, d, J = 8.85 Hz) and 7.33 (2H, d, J = 8.85 Hz).

Biphenyl acetate[20] **(10a):** White platelet (93%), m.p. 83-84°C (hexane) (lit[20] m.p. 88-89°C), R_f 0.70 (chloroform). IR (KBr, cm^{-1}): 3066, 2981, 1750, 1596, 1485, 1373, 1194 and 1018; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.31 (3H, s), 7.16 (2H, d, J = 8.55 Hz), 7.34 (1H, tt, J = 7.33, 1.22 Hz), 7.43 (2H, tt, J = 7.63, 1.52 Hz), 7.56 (2H, dd, J = 8.24, 1.22 Hz) and 7.58 (2H, d, J = 8.54 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.1 (1C, CH_3), 121.8

(2C, C-2), 127.1 (2C, C-2′), 127.3 (1C, C-4′), 128.1 (2C, (C-3), 128.8 (2C, C-3′), 139.0 (1C, C-4), 140.4 (1C, C-1′), 150.1 (1C, C-1) and 169.5 (1C, C=O).

Preparation of 2-Hydroxyacetophenones



Compound	R	R′
2b	CH_3	H
5b	OCH_3	H
6b	Cl	H
10b	Ph	H
11b	H	OCH_2Ph
12b	H	$O(CH_2)_3CH_3$
13b	H	$O(CH_2)_5CH_3$
14b	H	$O(CH_2)_7CH_3$
15b	H	$O(CH_2)_{11}CH_3$

General Procedure:

Fries Rearrangement (for **2b, 6b** and **10b**)

Phenyl acetate was mixed with anhydrous aluminium chloride. The reaction mixture was refluxed at 120°C for 2 hours. The mixture was worked up by slowly pouring into ice water and then adding the cooled 2 N hydrochloric acid. The mixture was stirred at 0°C for 30 minutes to precipitate the product, which was filtered off and washed with cold water.[21]

Alkylation (for **11b-15b**)

A mixture of 2,4-dihydroxyacetophenone, alkyl halide, potassium iodide and anhydrous potassium carbonate in acetone which was dried over calcium chloride, was refluxed for 4 hours. The reaction mixture was cooled, filtered and washed with acetone. The filtrate was evaporated to give the crude product.[22]

2-Hydroxy-5-methylacetophenone[20] **(2b):** Yellow needle (84%), m.p. 44-45°C (hexane) (lit[20] m.p. 50°C), R_f 0.67 (chloroform). IR (KBr, cm^{-1}): 3300-2600, 3070-3010, 2990-2850, 1650, 1500, 1350, 1250 and 1050; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.29 (3H, s),

2.59 (3H, s), 6.85 (1H, d, J = 8.52 Hz), 7.26 (1H, dd, J = 8.32, 2.02 Hz), 7.47 (1H, s) and 12.07 (1H, s).

5-Chloro-2-hydroxyacetophenone[20] **(6b):** Pale yellow needle (80%), m.p. 51-52° C (hexane) (lit[20] m.p. 55°C), R_f 0.70 (chloroform). IR (KBr, cm^{-1}): 3300-2650, 3070-3010, 2970-2880, 1650, 1480, 1350, 1200 and 1010; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.61 (3H, s), 6.92 (1H, d, J = 8.92 Hz), 7.40 (1H, dd, J = 8.80, 2.48 Hz), 7.67 (1H, d, J = 2.56 Hz) and 12.13 (1H, s).

2-Hydroxy-5-phenylacetophenone[23] **(10b):** Yellow crystal (13%), m.p. 49-50°C (hexane), R_f 0.72 (chloroform). IR (KBr, cm^{-1}): 3300-2500, 3032, 2927-2789, 1643, 1477, 1369, 1215 and 1022; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.69 (3H, s), 7.05 (1H, d, J = 8.66 Hz), 7.34 (1H, tt, J = 7.33, 1.22 Hz), 7.43 (2H, tt, J = 7.63, 1.52 Hz), 7.56 (2H, dd, J = 8.24, 1.22 Hz), 7.70 (1H, dd, J = 8.66, 2.29 Hz), 7.90 (1H, d, J = 2.28 Hz) and 12.26 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.7 (1C, CH_3), 118.9 (1C, C-3), 126.6 (1C, C-6), 126.7 (2C, C-2′), 127.2 (1C, C-4′), 128.9 (2C, C-3′), 129.0 (1C, C-1), 132.4 (1C, C-5), 135.4 (1C, C-4), 140.0 (1C, C-1′), 161.8 (1C, C-2) and 204.6 (1C, C=O).

4-Benzyloxy-2-hydroxyacetophenone[22] **(11b):** Orange platelet (80%), m.p. 103-104°C (methanol) (lit[22] m.p. 105-106°C), R_f 0.53 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3250-2600, 3032, 2943-2885, 1624, 1493, 1365, 1246 and 1134; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.54 (3H, s), 5.07 (2H, s), 6.50 (1H, s), 6.51 (1H, d, J = 7.12 Hz), 7.35-7.42 (5H, m), 7.62 (1H, d, J = 9.60 Hz) and 12.74 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.2 (3C, CH_3), 70.2 (2C, CH_2), 101.9 (1C, C-3), 108.1 (1C, C-5), 114.1 (1C, C-1), 127.5 (2C, C-2′), 128.3 (1C, C-4′), 128.7 (2C, C-3′), 132.4 (1C, C-6), 135.9 (1C, C-1′), 165.2 (1C, C-2), 165.2 (1C, C-4) and 202.6 (1C, C=O).

4-Butyloxy-2-hydroxyacetophenone[20] **(12b):** Colorless crystal (80%), m.p. 42-43° C (hexane) (lit[20] m.p. 43°C), R_f 0.59 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3300-2650, 3059, 2954-2889, 1631, 1477, 1377, 1250, 1192 and 1065; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.94 (3H, t, J = 7.32 Hz), 1.45 (2H, hext, J = 7.58 Hz), 1.74 (2H, quint, J = 7.09 Hz), 2.50 (3H, s), 3.95 (2H, t, J = 6.54 Hz), 6.38 (1H, d, J = 8.38 Hz), 6.41 (1H, s), 7.57 (1H, dd, J = 8.36, 0.74 Hz) and 12.70 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 13.7 (1C, C-4′), 19.1 (1C, C-3′), 26.1 (1C, CH_3), 31.0 (1C, C-2′), 68.0 (1C, C-1′), 101.3 (1C, C-3),

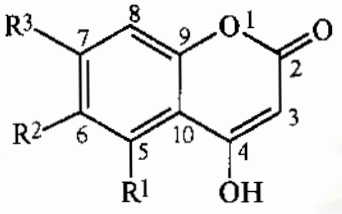
107.9 (1C, C-5), 113.7 (1C, C-1), 132.2 (1C, C-6), 165.2 (1C, C-2), 165.7 (1C, C-4) and 202.4 (1C, C=O).

4-Hexyloxy-2-hydroxyacetophenone[24] **(13b):** Yellow liquid (97%), R_f 0.56 (hexane/ethyl acetate 7:3). IR (neat, cm^{-1}): 3300-2600, 3078, 2954-2870, 1635, 1508, 1369, 1257, 1192 and 1068; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, t, J = 6.72 Hz), 1.25-1.33 (6H, m), 1.74 (2H, quint, J = 7.88 Hz), 2.49 (3H, s), 3.93 (2H, t, J = 6.56 Hz), 6.37 (1H, d, J = 8.58 Hz), 6.40 (1H, s), 7.56 (1H, d, J = 8.42 Hz) and 12.70 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.0(1C, C-6′), 22.5 (1C, C-5′), 25.6 (1C, C-3′), 26.1 (1C, CH_3), 28.9 (1C, C-2′), 31.5 (1C, C-4′), 68.4 (1C, C-1′), 101.3 (1C, C-3), 107.9 (1C, C-5), 113.7 (1C, C-1), 132.2 (1C, C-6), 165.2 (1C, C-2), 165.7 (1C, C-4) and 202.4 (1C, C=O).

2-Hydroxy-4-octyloxyacetophenone[24] **(14b):** Yellow liquid (77%), R_f 0.58 (hexane/ethyl acetate 7:3). IR (neat, cm^{-1}): 3300-2600, 3078, 2927-2858, 1635, 1508, 1369, 1261, 1192 and 1068; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, t, J = 6.70 Hz), 1.22-1.26 (10H, m), 1.75 (2H, quint, J = 7.86 Hz), 2.50 (3H, s), 3.94 (2H, t, J = 6.48 Hz), 6.38 (1H, d, J = 8.62 Hz), 6.41 (1H, s), 7.57 (1H, dd, J = 8.36, 0.64 Hz) and 12.70 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.0 (1C, C-8′), 22.6 (1C, C-7′), 25.9 (1C, CH_3), 26.1 (1C, C-3′), 28.9 (1C, C-5′), 29.2 (1C, C-4′), 29.3 (1C, C-2′), 31.8 (1C, C-6′), 68.4 (1C, C-1′), 101.3 (1C, C-3), 107.9 (1C, C-5), 113.7 (1C, C-1), 132.2 (1C, C-6), 165.2 (1C, C-2), 165.7 (1C, C-4) and 202.4 (1C, C=O).

4-Dodecyloxy-2-hydroxyacetophenone[24] **(15b):** White platelet (40%), m.p. 44-45° C (ethanol), R_f 0.60 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3300-2600, 3074, 2920-2850, 1643, 1577, 1365, 1253, 1196 and 1030; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, t, J = 6.18 Hz), 1.24-1.41 (18H, m), 1.76 (2H, quint, J = 7.72 Hz), 2.52 (3H, s), 3.95 (2H, t, J = 6.54 Hz), 6.39 (1H, d, J = 8.44 Hz), 6.42 (1H, s), 7.58 (1H, dd, J = 8.32, 0.62 Hz) and 12.71 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.1 (1C, C-12′), 22.7 (1C, C-11′), 25.9 (1C, CH_3), 26.1 (1C, C-3′), 28.9 (1C, C-9′), 29.3 (5C, C-4′, C-5′, C-6′, C-7′, C-8′), 29.6 (1C, C-2′), 31.9 (1C, C-10′), 68.4 (1C, C-1′), 101.3 (1C, C-3), 108.0 (1C, C-5), 113.7 (1C, C-1), 132.2 (1C, C-6), 165.3 (1C, C-2), 165.7 (1C, C-4) and 202.4 (1C, C=O).

Preparation of 4-Hydroxycoumarins



Compound	R^1	R^2	R^3
1	H	H	H
2	H	CH_3	H
3	OCH_3	H	H
4	H	OCH_3	H
5	H	H	OCH_3
6	H	Cl	H
7	H	Br	H
8	H	Br	OCH_3
9	H	CH_2CH_3	H
10	H	Ph	H
11	H	H	OCH_2Ph
12	H	H	$O(CH_2)_3CH_3$

General Procedure: Sodium hydride was added to a solution of 2-hydroxyacetophenone in diethyl carbonate. After initial vigorous reaction had subsided, the reaction mixture was refluxed for an hour. Ethanol was then added to destroy the excess of sodium hydride and the excess of diethyl carbonate was removed with ether. The product was obtained upon acidification. In the case of Compound **12b**, it was noted that this reaction provided 4-hydroxycoumarin as major product and non-cyclized compound as minor product. Whereas Compounds **13b-15b** were not cyclized to 4-hydroxycoumarin.[12,25]

4-Hydroxy-6-methyl-2H-1-benzopyran-2-one[7] **(2):** White crystal (87%), m.p. 247-248°C (ethanol) (lit[7] m.p. 261-264°C), R_f 0.60 (dichloromethane/ethanol 8.5:1.5). IR (KBr, cm^{-1}): 3700-2450, 3090-3010, 2940-2600, 1700, 1480, 1320, 1220 and 1100; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.35 (3H, s), 5.56 (1H, s), 7.24 (1H, d, J = 8.39 Hz), 7.43 (1H, dd, J = 8.57, 2.12 Hz), 7.59 (1H, s) and 12.49 (1H, br, s).

4-Hydroxy-6-methoxy-2H-1-benzopyran-2-one[7] **(4):** White needle (80%), m.p. 264-265°C (ethanol) (lit[7] m.p. 271-272°C), R_f 0.53 (dichloromethane/ethanol 8.5:1.5). IR

(KBr, cm^{-1}): 3250-2500, 3089, 2958-2565, 1689, 1554, 1300, 1277 and 1038; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.79 (3H, s), 5.58 (1H, s), 7.19 (1H, dd, J = 6.79, 3.03 Hz), 7.21 (1H, s), 7.30 (1H, d, J = 9.76 Hz) and 12.56 (1H, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 55.6 (1C, OCH_3), 91.2 (1C, C-3), 104.9 (1C, C-5), 116.2 (1C, C-10), 117.6 (1C, C-8), 120.3 (1C, C-7), 147.8 (1C, C-6), 155.2 (1C, C-9), 162.1 (1C, C-2) and 165.3 (1C, C-4).

6-Chloro-4-hydroxy-2H-1-benzopyran-2-one[7] **(6):** Pale yellow needle (99%), m.p. 248-249°C (ethanol) (lit[7] m.p. 266-268°C), R_f 0.68 (ethanol). IR (KBr, cm^{-1}): 3700-2450, 3100-3010, 2950-2600, 1700, 1620, 1580, 1300, 1200 and 1120; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.62 (1H, s), 7.43 (1H, d, J = 8.84 Hz), 7.69 (1H, dd, J = 8.96, 2.70 Hz), 7.77 (1H, d, J = 2.60 Hz) and 12.75 (1H, br, s).

4-Hydroxy-6-phenyl-2H-1-benzopyran-2-one[26] **(10):** White powder (19%), m.p. 249-250°C (ethanol), R_f 0.53 (dichloromethane/ethanol 8.5:1.5). IR (KBr, cm^{-1}): 3250-2500, 3065, 1689, 1600, 1242 and 1192; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.49 (1H, s), 7.40 (1H, t, J = 6.94 Hz), 7.44 (1H, d, J = 7.81 Hz), 7.47 (2H, t, J = 6.59 Hz), 7.69 (2H, d, J = 6.87 Hz), 7.89 (1H, d, J = 8.63, 2.34 Hz) and 8.05 (1H, d, J = 2.30 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 90.3 (1C, C-3), 116.9 (1C, C-8), 117.4 (1C, C-10), 121.0 (1C, C-5), 126.6 (1C, C-2′), 127.6 (1C, C-4′), 129.1 (2C, C-3′), 130.6 (1C, C-7), 135.6 (1C ,C-6), 138.9 (1C, C-1′), 153.2 (1C, C-9), 162.3 (1C, C-2) and 167.3 (1C, C-4).

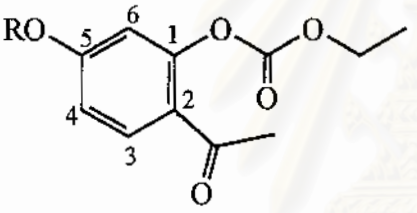
7-Benzyloxy-4-hydroxy-2H-1-benzopyran-2-one[22] **(11):** White powder (65%), m.p. 270-271°C (methanol) (lit[23] 272-273°C), R_f 0.63 (dichloromethane/ethanol 8.5:1.5). IR (KBr, cm^{-1}): 3300-2450, 3035, 2978-2588, 1682, 1628, 1308, 1246, 1165 and 1111; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.19 (2H, s), 5.44 (1H, s), 6.98 (1H, dd, J = 8.56, 2.39 Hz), 7.04 (1H, s), 7.32-7.49 (5H, m), 7.70 (1H, d, J = 8.72 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 69.8 (1C, CH_2), 88.5 (1C, C-3), 101.4 (1C, C-8), 112.4 (1C, C-6), 124.3 (1C, C-10), 127.9 (2C, C-2′), 128.0 (1C, C-4′), 128.1 (1C, C-5), 128.5 (2C, C-3′), 136.2 (1C, C-1′), 155.3 (1C, C-9), 161.9 (1C, C-7), 162.3 (1C, C-2) and 166.0 (1C, C-4)

7-Butyloxy-4-hydroxy-2H-1-benzopyran-2-one **(12):** White platelet (48%), m.p. 225-226°C (methanol), R_f 0.60 (dichloromethane/ethanol 8.5:1.5). IR (KBr, cm^{-1}): 3300-2500, 3105, 2958-2873, 1685, 1616, 1362, 1242, 1169; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.91 (3H, t, J = 7.22 Hz), 1.41 (2H, hext, J = 7.27 Hz), 1.71 (2H, quint, J = 6.33 Hz), 4.03

(2H, t, J = 6.43 Hz), 5.42 (1H, s), 6.87 (1H, dd, J = 6.46, 2.36 Hz), 6.91 (1H, d, J = 1.93 Hz), 7.67 (1H, dd, J = 6.38, 3.10 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.6 (1C, C-4′), 18.6 (1C, C-3′), 30.5 (1C, C-2′), 67.9 (1C, C-1′), 88.4 (1C, C-3), 100.9 (1C, C-8), 108.7 (1C,C-10), 112.1 (1C, C-6), 124.2 (1C, C-5), 155.4 (1C, C-9), 162.3 (1C, C-2), 162.3 (1C, C-7) and 166.0 (1C, C-4);); MS m/z (% rel. int.): 234 (M^+, 17), 178 (16), 150 (10), 136 (100) and 108 (21).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **12** are shown in Figs 1-4, respectively.

Non-cyclized compounds



Cpd	R
5c	CH_3
12c	$(CH_2)_3CH_3$
13c	$(CH_2)_5CH_3$
14c	$(CH_2)_7CH_3$
15c	$(CH_2)_{11}CH_3$

Ethyl-(2-acetyl-5-butyloxy)phenyl carbonate **(12c)**: White needle (12%), m.p. 133-134°C (methanol), R_f 0.53 (chloroform). IR (KBr, cm^{-1}): 3097, 2958-2873, 1747, 1618, 1560, 1419, 1331, 1223 and 1107; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.97 (3H, t, J = 7.32 Hz), 1.44 (3H, t, J = 7.11 Hz), 1.51-1.58 (2H, m), 1.80 (2H, quart, J = 8.13 Hz), 2.50 (3H, s), 4.02 (2H, t, J = 6.44 Hz), 4.47 (2H, quart, J = 7.13 Hz), 6.72 (1H, d, J = 2.33 Hz), 6.85 (1H, dd, J = 8.90, 2.41 Hz) and 7.88 (1H, d, J = 8.89 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 13.6 (1C, $OCH_2\underline{C}H_3$), 13.8 (1C, C-4′), 19.1 (1C, C-3′), 22.1 (1C, CH_3), 30.9 (1C, C-2′), 62.5 (1C, $O\underline{C}H_2CH_3$), 68.6 (1C, C-1′), 100.5 (1C, C-6), 107.2 (1C, C-2), 113.6 (1C, C-4), 126.4 (1C, C-3), 156.5 (1C, OC=O), 165.5 (1C, C-1), 166.7 (1C, C-5) and 171.6 (1C, C=O).

Ethyl-(2-acetyl-5-hexyloxy)phenyl carbonate **(13c)**: White needle (17%), m.p. 94-95°C (methanol), R_f 0.60 (chloroform). IR (KBr, cm^{-1}): 3082, 2958-2854, 1763, 1620,

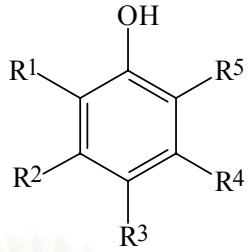
1558, 1419, 1389, 1227 and 1110; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86 (3H, t, J = 6.73 Hz), 1.30 (3H, t, J = 7.02 Hz), 1.39-1.43 (6H, m), 1.72 (2H, quint, J = 7.39 Hz), 2.50 (3H, s), 4.08 (2H, t, J = 6.48 Hz), 4.34 (2H, quart, J = 7.09 Hz), 6.95 (1H, d, J = 7.73 Hz), 6.99 (1H, s) and 7.83 (1H, d, J = 9.26 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.8 (1C, $OCH_2\underline{C}H_3$), 13.9 (1C, C-6′), 22.0 (2C, CH_3, C-5′), 25.0 (1C, C-3′), 28.3 (1C, C-2′), 30.9 (1C, C-4′), 61.8 (1C, $O\underline{C}H_2CH_3$), 68.6 (1C, C-1′), 100.7 (1C, C-6), 107.1 (1C, C-2), 113.3 (1C, C-4), 126.1 (1C, C-3), 156.0 (1C, OC=O), 164.8 (1C, C-1), 170.3 (1C, C-5) and 173.0 (1C, C=O).

Ethyl-(2-acetyl-5-octyloxy)phenyl carbonate **(14c)**: White needle (39%), m.p. 101-102°C (methanol), R_f 0.63 (chloroform). IR (KBr, cm^{-1}): 3086, 2920-2850, 1755, 1624, 1558, 1419, 1389, 1227 and 1107; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, t, J = 6.12 Hz), 1.20-1.37 (10H, m), 1.43 (3H, t, J = 7.12 Hz), 1.79 (2H, quint, J = 6.73 Hz), 2.50 (3H, s), 4.00 (2H, t, J = 6.43 Hz), 4.46 (2H, quart, J = 7.10 Hz), 6.70 (1H, d, J = 2.32 Hz), 6.83 (1H, dd, J = 8.84, 2.36 Hz) and 7.86 (1H, d, J = 8.86 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.1 (1C, $OCH_2\underline{C}H_3$), 14.2 (1C, C-8′), 22.6 (1C, CH_3), 25.9 (1C, C-7′), 28.8 (1C, C-3′), 29.2 (1C, C-5′), 29.3 (2C, C-2′, C-4′), 31.8 (1C, C-6′), 62.5 (1C, $O\underline{C}H_2CH_3$), 68.9 (1C, C-1′), 100.5 (1C, C-6), 107.4 (1C, C-2), 113.6 (1C, C-4), 126.3 (1C, C-3), 156.8 (1C, OC=O), 165.5 (1C, C-1), 172.2 (1C, C-5) and 175.5 (1C, C=O).

Ethyl-(2-acetyl-5-dodecyloxy)phenyl carbonate **(15c)**: White solid (40%), m.p. 89-90°C (methanol), R_f 0.64 (chloroform). IR (KBr, cm^{-1}): 3086, 2920-2850, 1755, 1624, 1558, 1419, 1389, 1223 and 1107; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, t, J = 6.09 Hz), 1.25-1.36 (18H, m), 1.43 (3H, t, J = 7.14 Hz), 1.80 (2H, quint, J = 6.69 Hz), 2.50 (3H, s), 4.01 (2H, t, J = 6.47 Hz), 4.47 (2H, quart, J = 7.10 Hz), 6.71 (1H, d, J = 2.33Hz), 6.84 (1H, dd, J = 8.86, 2.34 Hz) and 7.87 (1H, d, J = 8.85 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.1 (1C, $OCH_2\underline{C}H_3$), 14.2 (1C, C-12′), 22.7 (1C, CH_3), 25.9 (1C, C-11′), 28.8 (1C, C-3′), 29.3 (1C, C-9′), 29.5 (1C, C-2′), 29.6 (5C, C-4′, C-5′, C-6′, C-7′, C-8′), 31.9 (1C, C-10′), 62.5 (1C, $O\underline{C}H_2CH_3$), 68.9 (1C, C-1′), 100.5 (1C, C-6), 107.4 (1C, C-2), 113.6 (1C, C-4), 126.3 (1C, C-3), 156.8 (1C, OC=O), 165.5 (1C, C-1), 172.2 (1C, C-5) and 175.5 (1C, C-C=O).

2.3.2 Synthesis of Dicoumarols and Starting Materials

Formylation of Phenols



Compound	R^1	R^2	R^3	R^4	R^5
A1	CHO	H	OCH_3	H	H
A2	CHO	H	OCH_3	H	CHO
A3	CHO	H	CH_3	H	H
A4	CHO	H	CH_3	H	CHO
A5	CHO	H	CH_3	H	$C(CH_3)_3$
A6	CHO	H	$C(CH_3)_3$	H	H
A7	CHO	H	$C(CH_3)_3$	H	$C(CH_3)_3$
A8	CHO	H	OCH_2CH_3	H	$C(CH_3)_3$
A9	CHO	H	CHO	H	$C(CH_3)_3$

General Procedure: Acetic acid or trifluoroacetic acid was added to a mixture of phenol and hexamethylenetetramine. The solution was stirred and heated at 110°C for 2 hours. The reaction mixture was allowed to cool to 75°C, 33% (w/w) aqueous sulfuric acid was added and then the stirred mixture was heated at 110°C for an hour. After cooling down, the mixture was extracted with ether. The extract was washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*.[27]

2-Hydroxy-5-methoxybenzaldehyde[20] **(A1):** Yellow liquid (23%) (lit[20] b.p. 247-248°C), R_f 0.57 (hexane/ethyl acetate 9:1). IR (neat, cm^{-1}): 3500-3000, 3020, 2943, 2739, 1662, 1624, 1486, 1282, 1162 and 1048; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.77 (3H, s), 6.88 (1H, d, *J* = 9.04 Hz), 6.95 (1H, d, *J* = 3.02 Hz), 7.10 (1H, dd, *J* = 9.02, 3.12 Hz), 9.81 (1H, s), 10.61 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 55.9 (1C, OCH_3), 115.2 (1C, C-6), 118.7 (1C, C-3), 120.0 (1C, C-1), 125.2 (1C, C-4), 152.7 (1C, C-2), 156.0 (1C, C-5) and 196.1 (1C, C=O).

2-Hydroxy-5-methoxyisophthalaldehyde **(A2):** Yellow needle (11%), m.p. 135-136°C (hexane), R_f 0.47 (hexane/ethyl acetate 9:1). IR (KBr, cm^{-1}): 3500-3000, 3070, 2978, 2870, 1682, 1604, 1458, 1307, 1203 and 1049; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.84 (3H, s), 7.49 (2H, s), 10.20 (2H, s) and 11.10 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 56.2 (1C, OCH_3), 122.5 (2C, C-4, C-6), 123.5 (2C, C-1, C-3), 152.6 (1C, C-2), 158.0 (1C, C-5) and 191.8 (2C, C=O).

2-Hydroxy-5-methylbenzaldehyde[20] **(A3):** Yellow crystal (12%), m.p. 53-54°C (hexane) (lit[20] m.p. 56°C), R_f 0.66 (hexane/ethyl acetate 9:1). IR (KBr, cm^{-1}): 3500-3000, 3062, 2916, 2854, 1651, 1589, 1481, 1281, 1211 and 1149; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.32 (3H, s), 6.88 (1H, d, J = 9.08 Hz), 7.31 (1H, d, J = 8.87), 7.34 (1H, s), 9.83 (1H, s) and 10.81 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.2 (1C, CH_3), 117.4 (1C, C-3), 120.3 (1C, C-1), 129.1 (1C, C-5), 133.4 (1C, C-6), 138.0 (1C, C-4), 159.6 (1C, C-2) and 196.6 (1C, C=O).

2-Hydroxy-5-methylisophthalaldehyde **(A4):** Yellow needle (24%), m.p. 129-130°C (hexane/ethyl acetate), R_f 0.47 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3500-3000, 3032, 2924, 2870, 1682, 1604, 1458, 1304 and 1219; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.36 (3H, s), 7.74 (2H, s), 10.19 (2H, s) and 11.43 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.1 (1C, CH_3), 122.9 (2C, C-1, C-3), 129.5 (2C, C-4, C-6), 138.0 (1C, C-5), 161.8 (1C, C-2) and 192.2 (2C, C=O).

3-tert-Butyl-2-hydroxy-5-methylbenzaldehyde[27] **(A5):** Yellow crystal (12%), m.p. 71-72°C (hexane) (lit[27] m.p. 69-71°C), R_f 0.57 (hexane/ethyl acetate 9:1). IR (KBr, cm^{-1}): 3500-3000, 3086, 2962, 2870, 1651, 1466, 1321, 1272 and 1167; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.40 (9H, s), 2.31 (3H, s), 7.16 (1H, d, J = 2.14 Hz), 7.32 (1H, d, J = 2.18 Hz) 9.81 (1H, s) and 11.59 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.6 (1C, CH_3), 29.2 (3C, C($\underline{C}H_3$)$_3$), 34.7 (1C, $\underline{C}$(CH_3)$_3$), 120.4 (1C, C-1), 128.1 (1C, C-5), 131.4 (1C, C-6), 135.4 (1C, C-4), 138.0 (1C, C-3), 159.1 (1C, C-2) and 197.1 (1C, C=O).

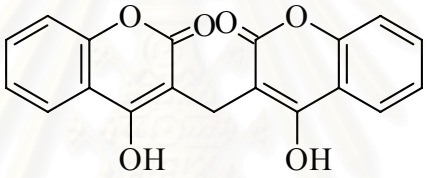
5-tert-Butyl-2-hydroxybenzaldehyde[20] **(A6):** Yellow liquid (7%) (lit[20] b.p. 251-252°C), R_f 0.54 (hexane/ethyl acetate 9:1). IR (neat, cm^{-1}): 3750-3000, 3028, 2966, 2870, 1658, 1593, 1485, 1377, 1265 and 1184; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.31 (9H, s), 6.92 (1H, d, J = 8.66 Hz), 7.50 (1H, d, J = 2.38 Hz), 7.57 (1H, dd, J = 8.64, 2.58 Hz), 9.87 (1H, s) and 10.87 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 31.3 (3C, C($\underline{C}H_3$)$_3$),

34.1 (1C, C(CH_3)$_3$), 117.2 (1C, C-3), 120.0 (1C, C-1), 129.8 (1C, C-6), 134.7 (1C, C-4), 142.8 (1C, C-5), 159.5 (1C, C-2) and 196.8 (1C, C=O).

5-tert-Butyl-4-hydroxyisophthalaldehyde[27] **(A9):** Yellow solid (24%), m.p. 48-49°C (hexane/ethyl acetate) (lit[27] m.p. 49.5-51.5°C), R_f 0.52 (hexane/ethyl acetate 7:3). IR (neat, cm^{-1}): 3500-3000, 3020, 2974, 2916, 1693, 1665, 1612, 1392, 1215 and 1147; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.42 (9H, s), 7.96 (1H, d, *J* = 1.98 Hz), 8.04 (1H, d, *J* = 2.04 Hz), 9.89 (1H, s), 9.96 (1H, s) and 12.37 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 30.0 (3C, C(CH_3)$_3$), 35.1 (1C, C(CH_3)$_3$), 120.3 (1C, C-3), 128.5 (1C, C-1), 133.8 (1C, C-2), 135.4 (1C, C-6), 139.9 (1C, C-5), 166.1 (1C, C-4), 189.9 (1C, C=O) and 196.8 (1C, C=O).

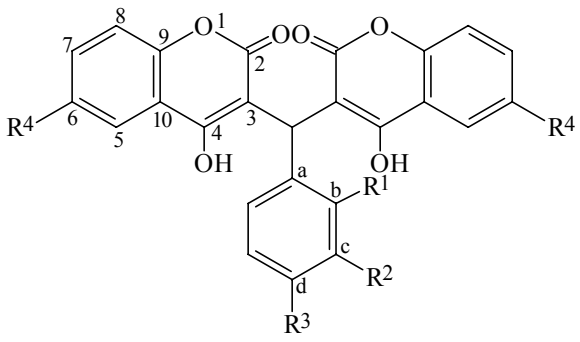
Preparation of Dicoumarols

3,3′-(Methylene)*bis*-4-hydroxycoumarin[12] **(D1)**



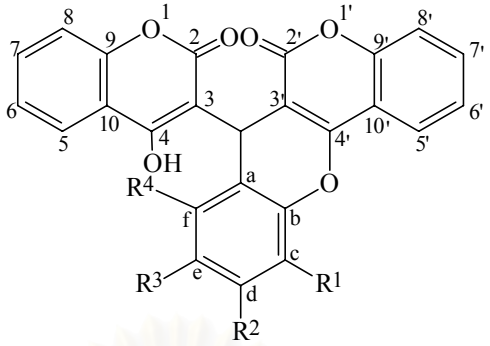
4-Hydroxycoumarin was dissolved in boiling water, the solution was allowed to cool to 70°C and 40% aqueous formaldehyde was quickly added with stirring. The mixture was then chilled, the crude product was filtered off, washed well with water and dried. The product **(D1)** was recrystallized from ethanol to give white solid (40%), m.p. 287-288°C (lit[12] m.p. 292-295°C), R_f 0.40 (ethyl acetate). IR (KBr, cm^{-1}): 3400-2500, 3066, 1652, 1597, 1567, 1501, 1451, 1346 and 1110; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.83 (2H, s), 7.33 (2H, d, *J* = 7.28 Hz), 7.39 (2H, t, *J* = 8.56 Hz), 7.58 (2H, dt, *J* = 7.97, 1.64 Hz), 7.98 (2H, dd, *J* = 8.68, 1.86 Hz) and 11.30 (2H, s).

Preparation of substituted Dicoumarols

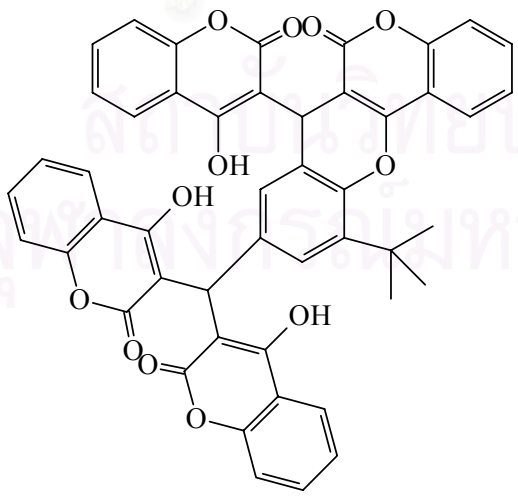


Compound	R^1	R^2	R^3	R^4
D2	H	H	H	H
D3	H	H	NO_2	H
D4	H	H	Cl	H
D5	H	H	F	H
D6	Br	H	H	H
D7	H	Br	H	H
D8	H	H	Br	H
D9	H	H	CH_3	H
D10	H	H	$CH(CH_3)_2$	H
D11	H	H	$C(CH_3)_3$	H
D12	H	H	CF_3	H
D13	OCH_3	H	H	H
D14	H	OCH_3	H	H
D15	H	H	OCH_3	H
D16	H	$-OCH_2O-$		H
D17	H	H	H	Cl
D18	H	H	NO_2	Cl
D19	H	H	OCH_3	Cl
D20	H	$-OCH_2O-$		Cl
D21	H	H	H	CH_3
D22	H	H	NO_2	CH_3
D23	H	H	OCH_3	CH_3
D24	H	$-OCH_2O-$		CH_3
D25	H	H	H	OCH_3
D26	H	H	NO_2	OCH_3
D27	H	H	OCH_3	OCH_3
D28	H	$-OCH_2O-$		OCH_3

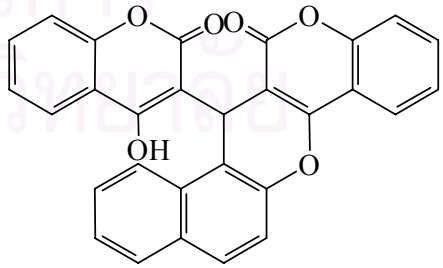
Preparation of Fused-ring compounds



Compound	R^1	R^2	R^3	R^4
D29	H	H	H	H
D30	OCH_3	H	H	H
D31	H	H	OCH_3	H
D32	CHO	H	OCH_3	H
D33	H	H	CH_3	H
D34	CHO	H	CH_3	H
D35	$C(CH_3)_3$	H	CH_3	H
D36	H	H	$C(CH_3)_3$	H
D37	$C(CH_3)_3$	H	$C(CH_3)_3$	H
D38	$C(CH_3)_3$	H	OCH_2CH_3	H
D40	H	H	H	Cl



(D39)



(D41)

General Procedure: 4-Hydroxycoumarin (2 mol-equivalent) was dissolved in ethanol and benzaldehyde was added. The solution was kept at that temperature for another 0.5 hour. After that the reaction mixture was cooled down. The product was filtered and washed with cold ethanol. In a case of Compound **D39**, 4 mol-equivalent of 4-hydroxycoumarin was used the same condition.[10]

3,3′-(Benzylidene)bis-4-hydroxycoumarin[28] **(D2):** White crystal (93%), m.p. 232-233°C (dichloromethane/ethanol) (lit[28] m.p. 227-229°C), R_f 0.76 (ethanol). IR (KBr, cm^{-1}): 3400-2500, 3020, 2850, 2750, 1650, 1500, 1460, 1350 and 1100; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.10 (1H, s), 7.22 (2H, d, *J* = 8.24 Hz), 7.27 (1H, t, *J* = 7.18 Hz), 7.32 (2H, t, *J* = 7.33 Hz), 7.37 (2H, br, t), 7.41 (2H, d, *J* = 8.24), 7.62 (2H, t, *J* = 7.63 Hz), 8.02 (2H, br, d), 11.28 (1H, s) and 11.51 (1H, s).

3,3′-(4-Nitrobenzylidene)bis-4-hydroxycoumarin[29] **(D3):** Yellow crystal (91%), m.p. 236-237°C (dichloromethane/ethanol) (lit[29] m.p. 234-236°C), R_f 0.76 (ethanol). IR (KBr, cm^{-1}): 3300-2500, 3090, 2950, 2720, 1650, 1500, 1350 and 1100; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.11 (1H, s), 7.40 (2H, d, *J* = 7.93, 1.07 Hz), 7.41 (2H, br, t), 7.42 (2H, d, *J* = 7.63 Hz), 7.66 (2H, t, *J* = 7.54), 7.99 (1H, d, *J* = 7.82 Hz), 8.08 (1H, d, *J* = 7.92 Hz), 8.18 (2H, d, *J* = 8.48 Hz), 11.36 (1H, s) and 11.55 (1H, s).

3,3′-(4-Chlorobenzylidene)bis-4-hydroxycoumarin[30] **(D4):** White crystal (81%), m.p. 251-252°C (dichloromethane/ethanol) (lit[30] m.p. 250-252°C), R_f 0.49 (ethyl acetate). IR (KBr, cm^{-1}): 3350-2550, 3080, 2990, 2880, 1675, 1600, 1560, 1350 and 1100; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.03 (1H, s), 7.14 (2H, d, *J* = 8.59 Hz), 7.28 (2H, dd, *J* = 6.49, 2.06 Hz), 7.38 (2H, t, *J* = 8.24 Hz), 7.41 (2H, d, *J* = 8.24 Hz), 7.63 (2H, dt, *J* = 7.83, 1.59 Hz), 7.98 (1H, d, *J* = 7.72 Hz), 8.05 (1H, d, *J* = 7.53 Hz), 11.30 (1H, s) and 11.52 (1H, s).

3,3′-(Benzylidene)bis-6-chloro-4-hydroxycoumarin[31] **(D17):** White crystal (95%), m.p. 179-180°C (dichloromethane) (lit[31] m.p. 212-214°C), R_f 0.50 (dichloromethane/ethanol 3:1). IR (KBr, cm^{-1}): 3300-2400, 3082, 2931, 2727, 2623, 1662, 1562, 1446, 1346, 1250 and 1180; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.04 (1H, s), 7.16 (2H, dd, *J* = 6.72, 1.23 Hz), 7.26 (1H, t, *J* = 6.72 Hz), 7.31 (2H, t, *J* = 7.63 Hz), 7.34 (2H, d, *J* = 8.85 Hz), 7.56 (2H, dd, *J* = 8.85, 2.45 Hz), 7.94 (1H, s), 8.01 (1H, s), 11.23 (1H, s) and 11.41 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.2 (1C, <u>C</u>H-Ar), 104.4, 106.2 (2×1C, C-3), 117.5, 117.9 (2×1C, C-10), 118.1 (2C, C-8), 123.9 (1C, C-d), 126.3 (2C, C-5), 127.1 (2C, C-c), 128.7 (2C, C-8), 130.6 (2C, C-6), 132.9 (2C, C-

b), 134.4 (1C, C-a), 150.5, 150.8 (2×1C, C-9), 163.4, 164.6 (2×1C, C-2) and 166.3, 168.8 (2×1C, C-4).

3,3′-(4-Nitrobenzylidene)bis-6-chloro-4-hydroxycoumarin **(D18):** Yellow crystal (39%), m.p. 263-264°C (ethyl acetate), R_f 0.46 (dichloromethane/ethanol 3:1). IR (KBr, cm^{-1}): 3300-2500, 3082, 2981-2723, 1658, 1566, 1356, 1300-1119; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.34 (1H, s), 7.33 (2H, d, *J* = 8.54 Hz), 7.38 (2H, dd, *J* = 8.85, 1.23 Hz), 7.55 (2H, dd, *J* = 8.54, 2.44 Hz), 7.76 (2H, d, *J* = 2.75) and 8.05 (2H, d, *J* = 8.85); ^{13}C-NMR (DMSO-d_6) δ (ppm): 36.8 (1C, CH-Ar), 103.5 (2C, C-3), 117.8 (2C, C-8), 120.8 (2C, C-10), 123.1 (2C, C-c), 123.2 (2C, C-5), 127.4 (2C, C-6), 127.9 (2C, C-7), 131.0 (2C, C-b), 145.5 (1C, C-a), 150.3 (1C, C-d), 151.1 (2C, C-9), 163.8 (2C, C-2) and 166.1 (2C, C-4); MS m/z (% rel. int.): 329 (4), 312 (13), 282 (10), 196 (27), 154 (100) and 126 (55).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D18** are shown in Figs 5-8, respectively.

3,3′-(4-Methoxybenzylidene)bis-6-chloro-4-hydroxycoumarin[31] **(D19):** White crystal (92%), m.p. 233-234°C (lit[43] m.p. 218-220°C), R_f 0.56 (dichloromethane/ethanol 3:1). IR (KBr, cm^{-1}): 3300-2500, 3074, 2981-2831, 1658, 1566, 1342, 1252 and 1180; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.78 (3H, s), 5.99 (1H, s), 6.83 (2H, d, *J* = 8.88 Hz), 7.06 (2H, d, *J* = 8.06 Hz), 7.34 (2H, d, *J* = 8.84 Hz), 7.55 (2H, dd, *J* = 8.74, 2.42 Hz), 7.98 (2H, s), 11.25 (1H, s) and 11.38 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.6 (1C, CH-Ar), 55.3 (1C, OCH_3), 104.8, 106.3 (2×1C, C-3), 114.1 (2C, C-c), 117.5, 117.8 (2×1C, C-10), 118.2 (2C, C-8), 123.9 (2C, C-5), 126.2 (1C, C-a), 127.5 (2C, C-7), 130.6 (2C, C-6), 132.9(2C, C-b), 150.7 (2C, C-9), 158.6 (1C, C-d), 163.4, 164.4 (2×1C, C-2) and 166.3, 168.7 (2×1C, C-4).

3,3′-(3,4-Methylenedioxybenzylidene)bis-6-chloro-4-hydroxycoumarin **(D20):** Pale yellow crystal (85%), m.p. 173-174°C, R_f 0.56 (dichloromethane/ethanol 3:1). IR (KBr, cm^{-1}): 3300-2500, 3078, 2912-2719, 1674, 1562, 1335 and 1254-1180: ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.93 (2H, s), 5.95 (1H, s), 6.60 (1H, d, *J* = 1.53 Hz), 6.61-6.62 (1H, m), 6.72 (1H, d, *J* = 8.55 Hz), 7.33 (2H, d, *J* = 8.85 Hz), 7.55 (2H, dd, *J* = 8.85, 2.45 Hz), 7.95 (1H, s), 8.00 (1H, s), 11.19 (1H, s) and 11.47 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.0 (1C, CH-Ar), 101.3 (1C, CH_2), 104.6, 106.3 (2×1C, C-3), 107.0 (1C, C-e), 108.3 (1C, C-b), 117.5, 117.9 (2×1C, C-10), 118.1 (2C, C-8), 119.5

(1C, C-f), 123.9 (2C, C-5), 128.2 (2C, C-6), 130.6 (1C, C-a), 133.0 (2C, C-7), 146.6 (1C, C-d), 148.3 (1C, C-c), 150.5, 150.8 (2×1C, C-9), 163.5, 164.6 (2×1C, C-2) and 166.3, 168.8 (2×1C, C-4); MS m/z (% rel. int.): 524 (M^+, 0.3), 328 (22), 196 (13), 173 (23), 154 (41), 145 (31), 126 (45) and 63 (100).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D20** are shown in Figs 9-12, respectively.

3,3′-(Benzylidene)bis-4-hydroxy-6-methylcoumarin[31] **(D21):** White crystal (73%), m.p. 235-237°C (dichloromethane/ethanol) (lit[31] m.p. 227°C), R_f 0.48 (ethyl acetate). IR (KBr, cm^{-1}): 3300-2500, 3059, 2974-2731, 1666, 1574, 1350, 1203 and 1097; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.44 (6H, s), 6.07 (1H, s), 7.17-7.27 (5H, m), 7.32 (2H, d, *J* = 6.74 Hz), 7.41 (2H, dd, *J* = 8.60, 2.02 Hz), 7.77 (1H, s), 7.83 (1H, s), 11.33 (1H, s) and 11.57 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (2C, CH_3), 36.2 (1C, CH-Ar), 103.8, 105.6 (2×1C, C-3), 116.1, 116.6 (2×1C, C-10), 116.4 (2C, C-8), 123.9 (1C, C-d), 126.5 (2C, C-5), 126.8 (2C, C-c), 128.6 (2C, C-7), 133.9 (2C, C-b), 134.7 (2C, C-6), 135.4 (1C, C-a), 150.5, 150.7 (2×1C, C-9), 164.6, 165.8 (2×1C, C-2) and 167.0, 169.4 (2×1C, C-4).

3,3′-(4-Nitrobenzylidene)bis-4-hydroxy-6-methylcoumarin **(D22):** White powder (57%), m.p. 270-272°C (dichloromethane), R_f 0.40 (ethyl acetate). IR (KBr, cm^{-1}): 3300-2500, 3070, 2927-2731, 1658, 1574, 1350 and 1203-1088; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.43 (3H, s), 2.46 (3H, s), 6.08 (1H, s), 7.31 (2H, d, *J* = 8.39 Hz), 7.37 (2H, dd, *J* = 8.85, 1.22 Hz), 7.44 (2H, d, *J* = 8.54 Hz), 7.77 (1H, s) 7.85 (1H, s), 8.16 (2H, d, *J* = 8.85), 11.36 (1H, s) and 11.58 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (1C, CH_3), 36.6 (1C, CH-Ar), 103.3, 104.8 (2×1C, C-3), 116.0, 116.4 (2×1C, C-10), 116.5, 116.6 (2×1C, C-8), 123.8 (2C, C-c), 124.0 (2C, C-5), 127.6 (2C, C-7), 134.4 (2C, C-b), 135.1, 135.2 (2×1C, C-6), 143.6 (1C, C-a), 146.9 (1C, C-d), 150.6, 150.9 (2×1C, C-9), 164.9, 166.4 (2×1C, C-2) and 167.1, 169.2 (2×1C, C-4); MS m/z (% rel. int.): 485 (M^+, 0.8), 309 (3.8), 292 (15), 262 (32), 176 (33), 134 (100), 106 (40) and 78 (34).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D22** are shown in Figs 13-16, respectively.

3,3′-(4-Methoxybenzylidene)bis-4-hydroxy-6-methylcoumarin[31] **(D23):** Pale yellow solid (62%), m.p. 200-202°C (dichloromethane/ethanol) (lit[31] m.p. 223°C), R_f

0.44 (ethyl acetate). IR (KBr, cm^{-1}): 3300-2500, 3066, 2947-2735, 1658, 1570, 1300, 1257 and 1084; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.43 (6H, s), 3.78 (3H, s), 6.01 (1H, s), 6.83 (2H, d, *J* = 8.86 Hz), 7.09 (2H, dd, *J* = 8.78, 0.88 Hz), 7.27 (2H, d, *J* = 8.48 Hz), 7.41 (2H, dd, *J* = 8.62, 2.22 Hz), 7.77 (1H, s), 7.82 (1H, s), 11.31 (1H, s) and 11.54 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (2C, CH_3), 35.5 (1C, CH-Ar), 55.3 (1C, OCH_3), 104.1, 105.7 (2×1C, C-3), 114.0 (2C, C-c), 115.6, 115.9 (2×1C, C-10), 116.3 (2C, C-8), 123.9 (2C, C-5), 127.1 (1C, C-a), 127.6 (2C, C-7), 133.8 (2C, C-b), 134.7 (2C, C-6), 150.7 (2C, C-9), 158.4 (1C, C-d), 164.5, 165.7 (2×1C, C-2) and 167.0, 169.4 (2×1C, C-4).

3,3′-(3,4-Methylenedioxybenzylidene)bis-4-hydroxy-6-methylcoumarin **(D24):** Pale yellow solid (56%), m.p. 225-227°C (dichloromethane/ethanol), R_f 0.46 (ethyl acetate). IR (KBr, cm^{-1}): 3300-2500, 3066, 2900-2773, 1658, 1570, 1341 and 1203-1045; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.41 (3H, s), 2.43 (3H, s), 5.91 (2H, s), 5.97 (1H, s), 6.63 (1H, d, *J* = 1.22 Hz), 6.64-6.65 (1H, m), 6.70 (1H, d, *J* = 8.55 Hz), 7.26 (2H, d, *J* = 7.94 Hz), 7.40 (2H, dd, *J* = 8.55, 2.14 Hz), 7.76 (1H, s), 7.81 (1H, s), 11.28 (1H, s) and 11.62 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (2C, CH_3), 35.9 (1C, CH-Ar), 101.1 (1C, CH_2), 103.9, 105.6 (2×1C, C-3), 107.2 (1C, C-e), 108.1 (1C, C-b), 116.0, 116.5 (2×1C, C-10), 116.3 (2C, C-8), 119.5 (1C, C-f), 123.9 (2C, C-5), 129.1 (1C, C-a), 133.8 (2C, C-7), 134.7 (2C, C-6), 146.3 (1C, C-d), 148.1 (1C, C-c), 150.4, 150.7 (2×1C, C-9), 164.5, 165.7 (2×1C, C-2) and 166.8, 169.3 (2×1C, C-4); MS m/z (% rel. int.): 484 (M^+, 0.5), 308 (36), 176 (48), 134 (100) and 106 (27).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D24** are shown in Figs 17-20, respectively.

3,3′-(Benzylidene)bis-4-hydroxy-6-methoxycoumarin **(D25):** White crystal (65%), m.p. 265-267°C (dichloromethane), R_f 0.71 (dichloromethane/ethanol 7:3). IR (KBr, cm^{-1}): 3300-2500, 3064, 2989-2845, 1651, 1574, 1351 and 1282-1092; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.79 (6H, s), 6.35 (1H, s), 7.12 (2H, d, *J* =7.48 Hz), 7.13 (1H, t, *J* = 6.72 Hz), 7.16 (2H, dd, *J* = 8.85, 3.05 Hz), 7.21 (2H, t, *J* =7.02 Hz), 7.30 (2H, d, *J* = 8.85 Hz) and 7.35 (2H, d, *J* = 3.05 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 36.0 (1C, CH-Ar), 55.6 (2C, OCH_3), 104.2 (2C, C-3), 105.6 (2C, C-5), 117.2 (2C, C-7), 118.6 (2C, C-10), 119.7 (2C, C-8), 125.4 (1C, C-d), 126.7 (2C, C-c), 128.0 (2C, C-b), 140.3 (1C, C-a), 146.6 (2C, C-9), 155.3 (2C, C-6), 164.9 (2C, C-2) and 165.4

(2C, C-4); MS m/z (% rel. int.): 472 (M^+, 0.5), 280 (66), 192 (40), 150 (100), 135 (21), 122 (24), 107 (76) and 79 (56).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D25** are shown in Figs 21-24, respectively.

3,3′-(4-Nitrobenzylidene)bis-4-hydroxy-6-methoxycoumarin **(D26):** Pale yellow crystal (71%), m.p. 263-265°C (dichloromethane/ethanol), R_f 0.67 (dichloromethane/ethanol 7:3). IR (KBr, cm^{-1}): 3300-2500, 3082, 2962-2606, 1658, 1574, 1351 and 1282-1043; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.85 (3H, s), 3.88 (3H, s), 6.09 (1H, s), 7.20 (2H, dd, *J* = 9.15, 2.75 Hz), 7.33 (2H, d, *J* = 9.16 Hz), 7.34 (1H, d, *J* = 3.05 Hz), 7.43 (1H, d, *J* = 3.05 Hz), 7.38 (2H, d, *J* = 7.94 Hz), 8.16 (2H, d, *J* = 8.85 Hz), 11.49 (1H, s) and 11.69 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.6 (1C, CH-Ar), 55.9, 56.0 (2×1C, OCH_3), 103.4, 104.9 (2×1C, C-3), 105.1, 105.2 (2×1C, C-5), 116.6, 117.1 (2×1C, C-10), 117.9, 118.0 (2×1C, C-7), 122.1, 122.2 (2×1C, C-8), 123.8 (2C, C-c), 127.5 (2C, C-b), 143.4 (1C, C-a), 146.8 (1C, C-d), 147.1 (2×1C, C-9), 156.7 (2C, C-6), 164.6, 166.2 (2×1C, C-2) and 167.0, 169.1 (2×1C, C-4); MS m/z (% rel. int.): 517 (M^+, 0.3), 325 (16), 278 (11), 192 (30), 150 (100) and 107 (27).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D26** are shown in Figs 25-28, respectively.

3,3′-(4-Methoxybenzylidene)bis-4-hydroxy-6-methoxycoumarin **(D27):** Pale yellow crystal (75%), m.p. 197-199°C (dichloromethane/ethanol), R_f 0.61 (dichloromethane/ethanol 8:2). IR (KBr, cm^{-1}): 3300-2500, 3070, 2966-2607, 1658, 1574, 1342 and 1257-1084; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.77 (3H, s), 3.83 (3H, s), 3.87 (3H, s), 6.02 (1H, s), 6.83 (2H, d, *J* = 8.85 Hz), 7.09 (2H, dd, *J* = 8.85, 1.22 Hz), 7.16 (2H, dd, *J* = 9.16, 3.05 Hz), 7.30 (2H, d, *J* = 8.70 Hz), 7.36 (1H, s), 7.42 (1H, s), 11.41 (1H, s) and 11.60 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.5 (1C, CH-Ar), 55.2, 55.8, 55.9 (3×1C, OCH_3), 104.3, 105.9 (2×1C, C-3), 105.0, 105.1 (2×1C, C-5), 113.9 (2C, C-7), 116.8, 117.3 (2×1C, C-10), 117.8 (2C, C-c), 121.5, 121.6 (2×1C, C-8), 127.0 (1C, C-a), 127.5 (2C, C-b), 146.7, 147.0 (2×1C, C-9), 156.5 (2C, C-6), 158.3 (1C, C-d), 164.3, 165.4 (2×1C, C-2) and 166.8, 169.3 (2×1C, C-4); MS m/z (% rel. int.): 310 (45), 279 (34), 192 (32), 150 (100), 135 (21), 107 (44) and 79 (31).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D27** are shown in Figs 29-32, respectively.

3,3′-(3,4-Methylenedioxybenzylidene)bis-4-hydroxy-6-methoxycoumarin **(D28):** Yellow crystal (70%), m.p. 258-260°C (dichloromethane), R_f 0.60 (dichloromethane/ethanol 8:2). IR (KBr, cm^{-1}): 3300-2500, 3074, 2966-2606, 1655, 1575, 1346 and 1252-1053; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.78 (6H, s), 5.92 (1C, CH_2), 6.21 (1H, s), 6.56 (1H, dd, *J* = 8.24, 1.22 Hz), 6.62 (1H, s), 6.72 (1H, d, *J* = 8.24 Hz), 7.13 (2H, dd, *J* = 8.85, 3.05 Hz), 7.26 (2H, d, *J* = 8.85 Hz) and 7.33 (2H, d, *J* = 3.05 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 35.9 (1C, CH-Ar), 55.5 (2C, OCH_3), 100.6 (1C, CH_2), 104.3 (2C, C-3), 105.6 (2C, C-5), 107.4 (2C, C-7), 107.6 (1C, C-e), 117.0 (1C, C-b), 119.1 (2C, C-10), 119.4 (2C, C-8), 119.5 (1C, C-f), 134.8 (1C, C-a), 144.9 (2C, C-9), 146.6 (1C, C-d), 147.1 (1C, C-c), 155.1 (2C, C-6), 164.7 (2C, C-2) and 165.9(2C, C-4); MS m/z (% rel. int.): 324 (25), 192 (30), 150 (100), 135 (16) and 107 (25).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D28** are shown in Figs 33-36, respectively.

3-[6-Oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin[11] **(D29):** White needle (57%), m.p. 251-253°C (dichloromethane) (lit[11] m.p. 252-254° C), R_f 0.44 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2500, 3090, 2970-2650, 1700, 1650, 1560, 1210, 1110 and 1070; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.73 (1H, s), 7.13-7.33 (4H, m), 7.34 (1H, dt, *J* = 8.24, 1.83 Hz), 7.38 (1H, d, *J* = 6.40 Hz), 7.45 (1H, d, *J* = 8.39 Hz), 7.49 (1H, dt, *J* = 7.63, 0.92 Hz), 7.60 (1H, dt, *J* = 7.78, 1.53 Hz), 7.70 (1H, dt, 7.78, 1.53 Hz), 8.01 (1H, br, d) and 8.09 (1H, dd, *J* = 7.77, 1.27 Hz).

3-[3-Methoxy-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin[11] **(D31):** White solid (45%), m.p. 274-276°C (dichloromethane) (lit[11] m.p. 287-288°C), R_f 0.44 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2500, 3078, 2954-2846, 1705, 1612, 1496, 1396, 1211 and 1072; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.72 (3H, s), 5.33 (1H, s), 6.60 (1H, d, *J* = 2.79 Hz), 6.82 (1H, dd, *J* = 8.96, 2.87 Hz), 7.18 (1H, d, *J* = 6.55), 7.39 (2H, d, *J* = 7.95 Hz), 7.45 (2H, t, *J* = 7.08 Hz), 7.61 (2H, dt, *J* = 7.78, 1.21 Hz), 8.02 (1H, d, *J* = 7.90 Hz), 8.11 (1H, d, *J* = 7.82 Hz) and 10.44 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 30.4 (1C, CH-Ar), 55.6 (1C, OCH_3), 99.4 (1C, C-3), 108.5 (1C, C-3′), 113.0 (1C, C-d), 114.1 (1C, C-f), 114.7 (1C, C-10), 116.3 (1C, C-c), 116.8 (1C, C-10′), 116.9 (1C, C-8), 117.1 (1C, C-8′), 122.2 (1C, C-6), 123.4 (1C, C-6′), 123.9 (1C, C-5), 124.3 (1C, C-5′), 125.0 (1C, C-a), 132.0 (1C, C-7), 132.7 (1C, C-

7′), 145.1 (1C, C-b), 152.0 (1C, C-9), 153.1 (1C, C-9′), 157.0 (1C, C-e), 158.9 (1C, C-4′), 166.3 (1C, C-4), 161.2 (1C, C-2) and 161.5 (1C, C-2′).

3-[5-Carboxaldehyde-3-methoxy-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin **(D32):** Yellow solid (31%), m.p. 238-240°C (dichloromethane), R_f 0.42 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2500, 3078, 2993-2607, 1658-1612, 1566 and 1319-1103; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.69 (3H, s), 6.23 (1H, s), 7.04 (1H, d, *J* = 3.36 Hz), 7.12 (1H, d, *J* = 3.06 Hz), 7.26 (2H, d, *J* = 6.72), 7.28 (2H, t, *J* = 7.63 Hz), 7.53 (2H, dt, *J* = 7.48, 1.53 Hz), 7.86 (2H, dd, *J* = 7.94, 1.22 Hz) and 9.92 (1H, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 32.6 (1C, CH-Ar), 55.3 (1C, OCH_3), 103.6 (2C, C-3, C-3′), 110.8 (1C, C-d), 115.8 (1C, C-f), 118.5 (2C, C-8, C-8′), 120.1 (2C, C-10, C-10′), 123.4 (2C, C-6, C-6′), 123.8 (2C, C-5, C-5′), 125.3 (1C, C-c),131.4 (2C, C-7, C-7′), 131.9 (1C, C-a), 151.6 (2C, C-9, C-9′), 152.2 (1C, C-b), 153.8 (1C, C-e), 163.7 (2C, C-2, C-2), 165.4 (2C, C-4, C-4′) and 196.6 (1C, C=O); MS m/z (% rel. int.): 324 (44), 307 (21), 202 (22), 162 (64), 120 (92), 92 (100), 77 (33) and 63 (66).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D32** are shown in Figs 37-40, respectively.

3-[3-Methyl-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin[32] **(D33):** White solid (39%), m.p. 274-276°C (dichloromethane/ethanol) (lit[32] m.p. 275-277°C), R_f 0.54 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2750, 3062, 2924, 1712, 1635, 1496, 1389, 1211 and 1072; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.21 (3H, s), 5.69 (1H, s), 6.98 (1H, s), 7.10 (1H, dd, *J* = 8.11, 2.05 Hz), 7.22 (1H, d, *J* = 8.29), 7.32 (2H, d, *J* = 6.24 Hz), 7.46 (1H, t, *J* = 8.13 Hz), 7.47 (1H, t, *J* = 7.70 Hz), 7.60 (1H, dt, *J* = 8.35, 1.49 Hz), 7.69 (1H, dt, *J* = 7.49, 1.73 Hz) and 8.07 (2H, dd, *J* = 7.78, 1.32 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 20.3 (1C, CH_3), 28.6 (1C, CH-Ar), 113.8 (1C, C-c), 116.0 (1C, C-3), 116.1 (1C, C-10), 116.2 (1C, C-3′), 116.5 (1C, C-10′), 122.6 (2C, C-8, C-8′), 124.0 (2C, C-6, C-6′), 124.5 (2C, C-5, C-5′), 128.6 (1C, C-f), 128.9 (2C, C-7, C-7′), 132.2 (1C, C-d), 134.5 (1C, C-a), 147.2 (1C, C-e), 151.9 (1C, C-9), 152.2 (1C, C-9′), 155.9 (1C, C-b), 160.4 (2C, C-2, C-2′), 160.6 (2C, C-4, C-4′).

3-[5-Carboxaldehyde-3-methyl-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin **(D34) :** Pale yellow solid (29%), m.p. 286-288°C

(dichloromethane), R_f 0.74 (dichloromethane/ethanol 8:2). IR (KBr, cm^{-1}): 3700-2500, 3070, 2985-2623, 1697-1635, 1566, 1396 and 1211-1080; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.27 (3H, s), 5.73 (1H, s), 7.29 (1H, d, *J* = 1.53), 7.32 (1H, d, *J* = 8.24 Hz), 7.36 (1H, br), 7.46 (1H, t, *J* = 8.24 Hz), 7.49 (1H, dt, *J* = 7.79, 0.91 Hz), 7.55 (1H, d, *J* = 1.83 Hz), 7.60 (1H, dt, *J* = 7.78, 1.52 Hz), 8.03 (1H, br), 8.24 (1H, dd, *J* = 7.94, 1.53 Hz) and 10.65 (1H, s); ^{13}C-NMR (DMSO-d_6) δ (ppm): 20.1 (1C, CH_3), 28.4 (1C, CH-Ar), 113.7 (1C, C-3), 116.2 (1C, C-10), 116.4 (1C, C-10′), 116.5 (1C, C-3′), 123.1 (1C, C-8), 123.3 (1C, C-8′), 123.5 (1C, C-c), 124.1 (2C, C-6, C-6′), 124.8 (2C, C-5, C-5′), 128.4 (1C, C-a), 132.4 (1C, C-d), 132.8 (2C, C-7, C-7′), 134.7 (1C, C-e), 135.2 (1C, C-f), 152.0 (1C, C-9), 152.3 (1C, C-9′), 155.7 (1C, C-b), 160.3 (2C, C-2, C-2′), 161.2 (2C, C-4, C-4′) and 189.2 (1C, C=O); MS m/z (% rel. int.): 452 (M^+, 60), 331 (32), 317 (72), 304 (40), 291 (100), 120 (23), 92 (40) and 77 (51).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D34** are shown in Figs 41-44, respectively.

3-[5-tert-Butyl-3-methyl-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin **(D35) :** White crystal (30%), m.p. 271-273°C (ethanol), R_f 0.51 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2500, 3078, 2954-2870, 1716-1651, 1562, 1389 and 1207-1058: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.60 (9H, s), 2.23 (3H, s), 5.31 (1H, s), 6.77 (1H, d, *J* = 2.14 Hz), 7.07 (1H, d, *J* = 1.83), 7.15 (1H, d, *J* = 7.32 Hz), 7.27 (1H, dt, *J* = 7.32, 1.22 Hz), 7.40 (1H, d, *J* = 8.54 Hz), 7.45 (1H, dt, *J* = 7.02, 1.52 Hz), 7.46 (1H, dt, *J* = 7.53, 1.52 Hz), 7.61 (1H, dt, *J* = 7.79, 1.52 Hz), 8.01 (1H, dd, *J* = 7.94, 1.22 Hz), 8.16 (1H, dd, *J* = 8.24, 1.53 Hz) and 10.36 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.0 (1C, CH_3), 30.1 (1C, $C(CH_3)_3$), 30.2 (3C, $C(CH_3)_3$), 34.9 (1C, CH-Ar), 99.8 (1C, C-3), 109.1 (1C, C-3′), 115.0 (1C, C-10), 116.2 (1C, C-8), 116.9 (1C, C-10′), 117.2 (1C, C-8′), 121.1 (1C, C-a), 123.3 (1C, C-d), 123.7 (1C, C-6), 124.3 (1C, C-6′), 125.1 (1C, C-5), 127.0 (1C, C-5′), 127.1 (1C, C-f), 131.8 (1C, C-7), 132.5 (1C, C-7′), 134.3 (1C, C-e), 136.7 (1C, C-c), 147.9 (1C, C-b), 152.1 (1C, C-9), 153.0 (1C, C-9′), 158.4 (1C, C-4′), 160.9 (1C, C-2), 161.1 (1C, C-2′), 166.1 (1C, C-4); MS m/z (% rel. int.): 480 (M^+, 23), 319 (100), 303 (23), 162 (4), 120 (9) and 92 (14).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D35** are shown in Figs 45-48, respectively.

3-[3-tert-Butyl-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin **(D36) :** White crystal (80%), m.p. 262-263°C (ethyl acetate), R_f 0.57 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2500, 3070, 2954-2870, 1712-1612, 1566, 1396 and 1227-1068; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.18 (9H, s), 5.71 (1H, s), 7.20 (1H, s), 7.25 (1H, d, *J* = 8.55 Hz), 7.30 (1H, d, *J* = 7.93 Hz), 7.33 (1H, dd, *J* = 8.85, 2.44 Hz), 7.42 (1H, d, *J* = 8.55 Hz), 7.45 (2H, t, *J* = 7.79 Hz), 7.57 (1H, dt, *J* = 7.78, 1.52 Hz), 7.67 (1H, dt, *J* = 7.94, 1.83 Hz) and 8.07 (2H, dd, *J* = 7.93, 1.83 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 29.0 (1C, $\underline{C}(CH_3)_3$), 31.0 (3C, $C(\underline{C}H_3)_3$), 34.0 (1C, $\underline{C}$H-Ar), 113.9 (1C, C-3), 115.8 (1C, C-3′), 116.1 (1C, C-10), 116.2 (1C, C-10′), 116.5 (1C, C-c), 121.4 (1C, C-a), 122.6 (2C, C-8, C-8′), 123.9 (1C, C-d), 124.5 (2C, C-6, C-6′), 124.7 (1C, C-e), 125.4 (1C, C-f), 132.2 (2C, C-5, C-5′), 132.4 (2C, C-7, C-7′), 147.2 (1C, C-9), 147.7 (1C, C-9′), 152.0 (1C, C-2), 152.2 (1C, C-2′), 156.5 (1C, C-b), 160.4 (2C, C-4, C-4′); MS m/z (% rel. int.): 466 (M^+, 72), 318 (19), 305 (100), 289 (25), 275 (20), 162 (4) and 121 (36).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D36** are shown in Figs 49-52, respectively.

3-[3,5-Di-tert-butyl-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin **(D37) :** White crystal (59%), m.p. 246-248°C (ethanol), R_f 0.74 (dichloromethane/methanol 9:1). IR (KBr, cm^{-1}): 3750-2500, 3074, 2962-2870, 1720-1651, 1562, 1389 and 1207-1049; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.20 (9H, s), 1.55 (9H, s), 5.70 (1H, s), 7.10 (1H, d, *J* = 1.84 Hz), 7.25 (1H, d, *J* = 2.13 Hz), 7.28 (1H, d, *J* = 8.24 Hz), 7.34 (1H, t, *J* = 7.63 Hz), 7.46 (1H, d, *J* = 8.24 Hz), 7.54 (1H, dt, *J* = 7.63, 1.22 Hz), 7.58 (1H, dt, *J* = 7.78, 1.52 Hz), 7.70 (1H, dt, *J* = 7.79, 1.53 Hz) 8.02 (1H, br) and 8.05 (1H, dd, *J* = 7.94, 1.22 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 29.2 (1C, $\underline{C}(CH_3)_3$), 30.1 (3C, $C(\underline{C}H_3)_3$), 31.1 (3C, $C(\underline{C}H_3)_3$), 34.8 (1C, $\underline{C}$H-Ar), 107.0 (1C, C-3), 108.0 (1C, C-3′), 114.2 (1C, C-10), 116.2 (1C, C-10′), 116.9 (1C, C-d), 122.3 (2C, C-8, C-8′), 122.5 (1C, C-f), 123.2 (2C, C-6, C-6′), 123.9 (1C, C-a), 124.8 (2C, C-7, C-7′), 132.2 (1C, C-c), 132.4 (2C, C-5, C-5′), 135.9 (1C, C-e), 146.0 (1C, C-9), 146.7 (1C, C-9′), 152.1 (1C, C-2), 152.2 (1C, C-2′), 156.3 (1C, C-b), 160.5 (1C, C-4′), 165.4 (1C, C-4); MS m/z (% rel. int.): 522 (M^+, 3.9), 361 (68), 347 (44), 333 (34), 207 (20), 162 (39), 120 (61), 92 (53) and 57 (100).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D37** are shown in Figs 53-56, respectively.

3-[3-Ethoxy-5-tert-butyl-6-oxo(1)benzopyrano(4,3-b)-(1)benzopyran-7-yl]-4-hydroxycoumarin **(D38) :** White powder (36%), m.p. 260-262°C (dichloromethane/ethanol), R_f 0.54 (ethyl acetate). IR (KBr, cm^{-1}): 3700-2500, 3086, 2970-2870, 1712-1604, 1558, 1389 and 1203-1065; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.32 (3H, t, *J* = 7.02 Hz), 1.58 (9H, s), 3.90 (2H, quart, *J* = 6.71 Hz), 5.32 (1H, s), 6.43 (1H, d, *J* = 2.74 Hz), 6.84 (1H, d, *J* = 3.05), 7.16 (1H, d, *J* = 7.94 Hz), 7.27 (1H, t, *J* = 7.94 Hz), 7.41 (1H, d, *J* = 8.55 Hz), 7.45 (1H, t, *J* = 7.79 Hz), 7.46 (1H, dt, *J* = 8.24, 1.83 Hz), 7.62 (1H, dt, *J* = 7.78, 1.53 Hz), 8.00 (1H, dd, *J* = 7.94, 1.53 Hz), 8.14 (1H, dd, *J* = 7.94, 1.53 Hz) and 10.41 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.8 (1C, $OCH_2\underline{C}H_3$), 30.1 (3C, $C(\underline{C}H_3)_3$), 30.7 (1C, $\underline{C}(CH_3)_3$), 35.1 (1C, $\underline{C}$H-Ar), 63.7 (1C, $O\underline{C}H_2CH_3$), 99.2 (1C, C-3), 108.9 (1C, C-3′), 110.5 (1C, C-d), 113.8 (1C, C-f), 115.0 (1C, C-10), 116.2 (1C, C-8), 116.9 (1C, C-10′), 117.2 (1C, C-8′), 122.1 (1C, C-a), 123.3 (1C, C-6), 123.8 (1C, C-6′), 124.3 (1C, C-5), 125.1 (1C, C-5′), 131.8 (1C, C-7), 132.5 (1C, C-7′), 138.6 (1C, C-c), 144.1 (1C, C-b), 152.1 (1C, C-9), 153.0 (1C, C-9′), 155.6 (1C, C-e), 158.5 (1C, C-4′), 161.0 (1C, C-2), 161.1 (1C, C-2′) and 166.3 (1C, C-4); MS m/z (% rel. int.): 510 (M^+, 1.1), 350 (100), 349 (89), 335 (20), 162 (12), 120 (29) and 92 (56).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D38** are shown in Figs 57-60, respectively.

3,3′,3″,3‴-(5-tert-butyl-6-oxy-isophthalalidene)tetrakis[4-hydroxycoumarin] **(D39) :** White powder (36%), m.p. 244-246°C (dichloromethane/ethanol), R_f 0.52 dichloromethane/methanol 8.5:1.5). IR (KBr, cm^{-1}): 2750-2500, 3074, 2958-2603, 1716-1625, 1566, 1392 and 1215-1103; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.42 (9H, s), 5.58 (1H, s), 6.24 (1H, s), 6.88 (1H, s), 7.04 (1H, d, *J* = 1.52 Hz), 7.12 (1H, t, *J* = 7.93 Hz), 7.28 (1H, t, *J* = 7.93 Hz), 7.29 (1H, t, *J* = 7.94 Hz), 7.31 (1H, t, *J* = 6.86 Hz), 7.27 (1H, d, *J* = 7.94 Hz), 7.35 (1H, d, *J* = 8.24 Hz), 7.44 (1H, d, *J* = 8.24 Hz), 7.49 (1H, d, *J* = 7.33 Hz), 7.51 (1H, br), 7.52 (1H, t, *J* = 7.93 Hz), 7.58 (1H, dt, *J* = 8.55, 2.44 Hz), 7.58 (1H, dt, *J* = 6.87, 2.74 Hz), 7.68 (1H, dt, *J* = 7.79, 1.53 Hz), 7.78 (1H, br), 7.87 (1H, dd, *J* = 7.94, 1.53 Hz), 8.02 (1H, dd, 7.94, 1.22 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 29.9 (3C, $C(\underline{C}H_3)_3$), 34.5 (1C, $\underline{C}$H-Ar), 35.7 (1C, $\underline{C}(CH_3)_3$), 103.4 (3C, C-3), 104.1 (1C, C-3′), 114.2 (3C, C-8), 115.7, 116.8, 117.8 (3×1C, C-10), 116.0 (1C, C-8′), 118.1 (1C, C-10′), 122.3 (1C, C-d), 123.5, 123.7 (3×1C, C-6), 124.8 (1C, C-6′), 123.7, 123.8, 123.9 (3×1C, C-5), 124.0 (1C, C-5′), 125.4 (1C, C-a), 131.7, 131.9 (3×

1C, C-7), 132.4 (1C, C-7′), 132.0 (1C, C-f), 135.3 (1C, C-e), 135.7 (1C, C-c), 152.0, (3×1C, C-9), 152.1 (1C, C-9′), 152.2 (1C, C-b), 164.6 (3×1C, C-2), 164.9 (1C, C-2′), 165.3, 165.8 (3×1C, C-4) and 160.5 (1C, C-4′); MS m/z (% rel. int.): 314 (0.3), 207 (12), 162 (17), 120 (27) and 94 (100).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **D39** are shown in Figs 61-64, respectively.

Moreover, Compounds **1**, **3**, **5**, **7-9**, **A7-A8**, **D5-D16**, **D30**, **D40** and **D41** were kindly supplied by S. Wattanasereekul, W. Sirisuksukon and P. Boonsong.

2.4 Bioassay Procedures

This research brings the object into the study of substituents effect of 4-hydroxycoumarins and their analogues on their bioactivities. The bioassays, which were selected to perform in this research, are brine shrimp cytotoxic lethality test, weed growth inhibition test, static acute toxicity bioassay and antibacterial bioassay. All bioassay experiments are described in 2.4.1-2.4.4.

2.4.1 Brine Shrimp Cytotoxic Lethality Test[33]

There are many procedures for biological activity tests but they can be quite complicated and expensive. On the other hand, brine shrimp cytotoxic lethality test, a procedure for general toxicity screening, is rapid, reliable, convenient and inexpensive. Consequently, this procedure is utilizable for a preliminary testing in the study of bioactive compounds. A microwell method that is the one of techniques for this bioassay was used in this research.

a) Sample Preparation

Tested compound (4 mg) was dissolved in 80 μL of dimethyl sulfoxide (DMSO) and then artificial sea water (38 g of NaCl dissolved in 1 L of deionized or distilled water) was added to the solution to make 4000 μL affording solution A (1000 ppm). This stock solution was diluted to obtain solution B (100 ppm) and solution C (10 ppm), respectively. Control solution was prepared using only DMSO and artificial sea water.

b) Hatching the Shrimp

Brine Shrimp eggs (*Artemia salina* Leach.) were hatched in an open shallow rectangular plastic box (13×18×4 cm). The box was divided into two unequal

compartments linked with 2 mm ϕ holes and filled with artificial sea water. The eggs were sprinkled into the larger compartment which was darkened with aluminum foil whereas the smaller was illuminated with the 25-watt lamp. The box was kept at 22-29°C for 24 hours. The shrimps were then collected by disposable pipette from the lighted compartment.

c) Bioassay

Five shrimps were transferred to each sample well of 24-well microplates using the disposable pipette, and artificial sea water was added to make 100 μL. Each concentration was prepared in six replications. The covered plates were set in the same condition as hatching. After 24 hours, numbers of dead shrimp were counted under binocular microscope.

d) LC_{50} Determination

Probit analysis program was used to calculate LC_{50} values. In some cases where data was insufficient for this program, LC_{50} values were estimated using logic transformation.

2.4.2 Weed Growth Inhibition Test[34]

Tested compound was dissolved in a proper solvent[a] at concentration of 1000, 100, 10 and 1 ppm. The 3.0 mL of solution was poured into a glass tube (diameter 30 mm and length 120 mm) which contained 1.5 g of cellulose powder. The controlled tube was prepared by the same solvent using the same methodology. All tubes were covered with aluminum foil, dried up and heated at 50°C in vacuum oven for 6-12 hours, followed by the addition of 4.5 mL of distilled water to each tube and then cellulose powder was well-mixed. Three seedlings of giant mimosa with radical root length 1-2 mm (seed was previously grown for 3 days) were transplanted in each tube, 3 tubes for each concentration. The tubes were sealed with transparent vinyl film and kept in growth chamber at 30°C, 24 hours daylight. After 7 days, the seedlings were cleared from artificial food, both lengths of root and shoot of both treatment and controlled plants were measured and compared.[b]

---

a) ethanol for most 4-hydroxycoumarins (For Compound **11**, methanol was used)

b) All of these results were performed at Weed Science Sub-Division, Botany and Weed Science Division, Department of Agriculture, Minister of Agriculture and Cooperatives.

%Growth Inhibition = {1-(T/C)}×100%

where "T" is root (or shoot) length of treated seedlings and "C" is root (or shoot) length of controlled seedlings.

Growth inhibition of 100% represents total inhibition of growing.

2.4.3 Static Acute Toxicity Bioassay[35]

Acute toxicity test is an obvious and easily observed effect, which is widely used for evaluating of the chemical toxicity in the early period. The results of test are usually revealed as the concentration, which is 50% lethality (LC_{50}) of test organism during the particular time. In primary biological search, small vertebrates that were selected to study are fish such as Silver carp, Nile tilapia. Nile tilapia (*Oreochromis niloticus*) was used to experiment in this research because they are widely available and abundant in Thailand. The procedure of this test is described below.

a) Preparation of Experimental Fish

Nile tilapia were obtained from National Aquaculture Genetics Research Institute, Department of Fisheries, the Ministry of Agriculture and Cooperatives of Thailand. The fish (at the age of 2 weeks) were initially acclimated and observed in 325 L glass aquaria for 2 weeks prior to exposure. The fish were fed commercial pellets twice daily.

b) Sample Preparation

Samples were prepared by dissolving 1.6665 g of tested compound in 1.6665 mL of DMSO in the experimental chambers. Filtered water was then added to the solution to make 16.67 L affording solution A (100 ppm). Serial dilution of this stock solution was made to give solution B (10 ppm), solution C (1 ppm) and solution D (0.1 ppm), respectively. Control solution was prepared using only DMSO and filtered water. Each of concentration and control were performed to three replications.

c) Test Procedure

Six fish aged 30 days were randomly put into each chamber filled with 5 L of prepared sample. The fish were not fed 24 hours before starting and 96 hours during the experiment. The numbers of dead fish were recorded very 24 hour from the beginning until the end of the experiment (96 hours).

d) Data Analysis

LC_{50} values were calculated by probit analysis program.

2.4.4 Antibacterial Bioassay[*]

This bioassay was carried out by paper disc method, unless otherwise stated. The compounds were tested with seven bacterias: *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella derby*, *Escherichia coli* and Flat Sour Organisms. Stock solution was prepared by dissolving 10 mg of test sample in 1000 μL of proper solvent. 30 μL of stock solution were transferred by disposable pipette onto a disc. After 24 hours, diameter of clear zone was measured.

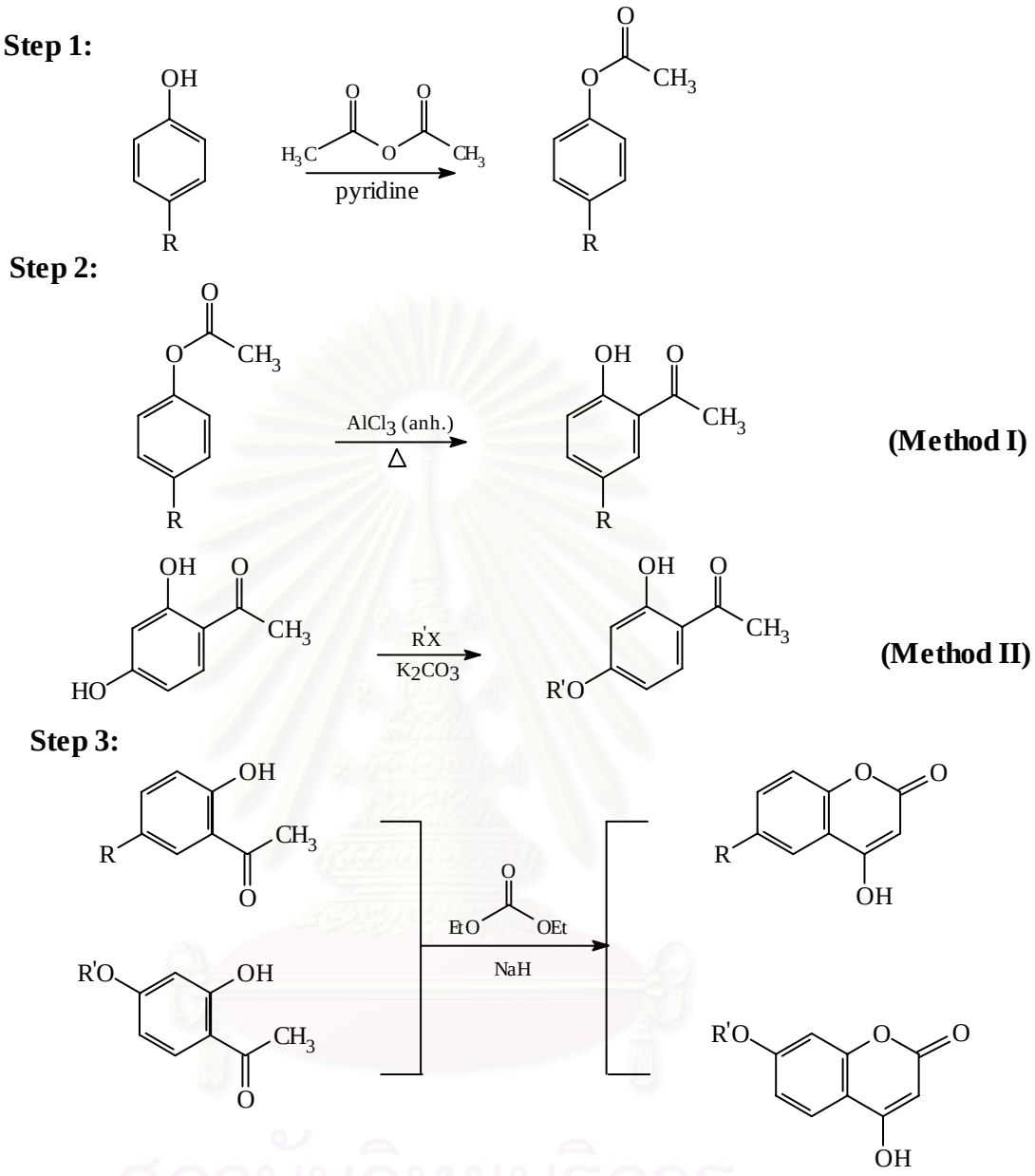
[*] This assay was performed by Ms. Siriporn Stonasaovapak at Institute of Food Research and Product Development, Kasetsart University.

CHAPTER III
RESULTS AND DISCUSSION

This research focussed on the syntheses and structure-activity relationship study of 4-hydroxycoumarins and their analogues. The position and type of substituents have markedly affected on biological activities. Four bioassays, namely brine shrimp cytotoxic lethality test, weed growth inhibition test, static acute toxicity bioassay and antibacterial bioassay have been evaluated.

3.1 Synthesis of 4-Hydroxycoumarins

Numerous methods to synthesize 4-hydroxycoumarins have been reported in literature. In this research, the syntheses of 4-hydroxycoumarins comprise three steps from precursors (Scheme 3.1). The first step (step 1) is acetylation of phenols with acetic anhydride in pyridine. Phenyl acetates were generally obtained in high yield (84-93%). The preparation of substituted 2-hydroxyacetophenone as a next step (step 2) is accomplished by utilizing two routes depending on starting materials. Method I involves Fries rearrangement to transform phenyl acetates into 2-hydroxyacetophenones. The other method (Method II) is the alkylation of 2,4-dihydroxyacetophenones with alkyl halide. Both methods gave products in moderate to high yield (40-97%) except for the yield of **10b** (13%). The final step (step 3) is the condensation of 2-hydroxyacetophenones with diethyl carbonate in the presence of sodium hydride following by *in situ* cyclization. Five desired products (**2, 4, 6, 10** and **11**) were achieved in moderate to high yield (48-99%) except for the yield of **10** (19%).

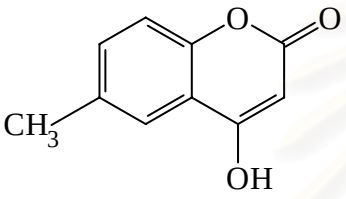


Scheme 3.1 General procedure for the synthesis of 4-hydroxycoumarins

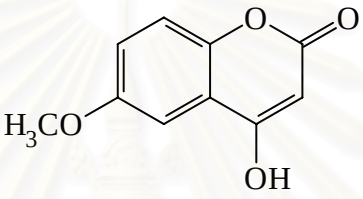
It should also be noted that under the conditions employed for cyclization 2-hydroxy-4-methoxyacetophenone **(5b)** and 4-butyloxy-2-hydroxyacetophenone **(12b)** afforded the cyclized products (**5**, 79% and **12**, 48%) together with a small amount of the non-cyclized products (**5c**, 21% and **12c**, 12%). However, in the case of Compounds **13b-15b**, the desired products were not obtained. These compounds (**5b**, **12b-15b**) contained alkoxy groups which were perhaps extended the conjugation length of the alkyl chain at

para position of acyl group, the resonance effect was thence occurred. As a result, they were converted to intermediates (**5c**, **12c-15c**) which were stable and less reactive with sodium hydride, low yield of the desired products were thus obtained (8-34%).

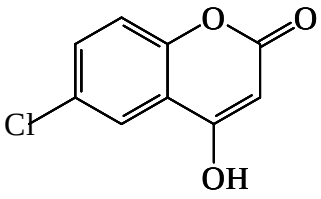
Twelve 4-hydroxycoumarins were synthesized. One of them, Compound **12**, has never been reported in chemical literature. The comparative results of the synthetic compounds in this research are tabulated in Table 3.1 and their structures are shown below.



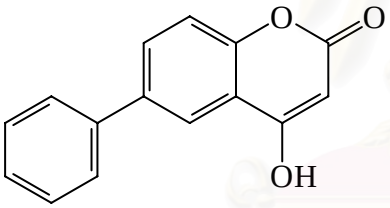
2



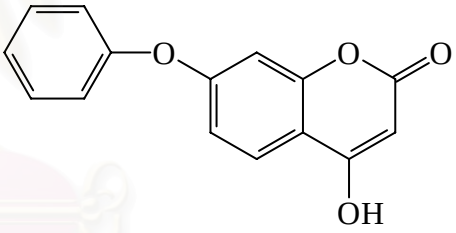
4



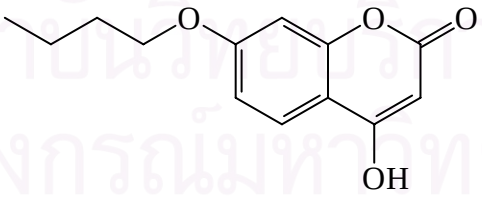
6



10



11



12

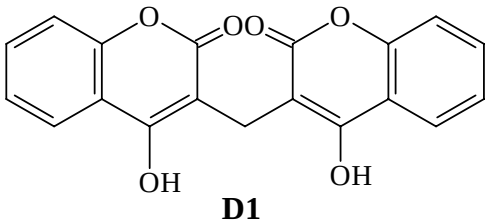
Table 3.1 Physical properties and %yield of synthesized 4-hydroxycoumarins

Cpd	Physical Properties		%Yield	References
	Appearance	m.p. (°C)		
2	white crystal	247-248	87	7
4	white needle	264-265	80	7
6	pale yellow needle	248-249	99	7
10	white powder	249-250	19	26
11	white powder	270-271	65	22
12	white platelet	225-226	48	new compound

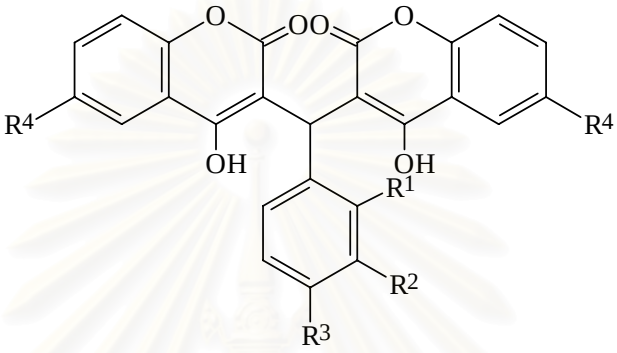
3.2 Synthesis of Dicoumarols and Their Analogues

The condensation between two-mole equivalents of 4-hydroxycoumarins and one mole equivalent of interested aromatic aldehydes is one of the methodologies for the preparation of dicoumarols. This method is convenient to perform and gives the corresponding products in excellent yield. Three procedures were carried out depending upon types of aldehydes. The first one was the condensation of 4-hydroxycoumarins with aromatic aldehydes in hot ethanol. The next procedure was used for formaldehyde which was rapidly reacted with 4-hydroxycoumarin in hot water. The last one was the same as the first procedure, but using *ortho*-hydroxy aromatic aldehydes as a reactant. These aromatic aldehydes were prepared by formylation of phenols with hexamethylenetetramine in acid conditions. This procedure gave the analogous products and can be called as fused compounds.

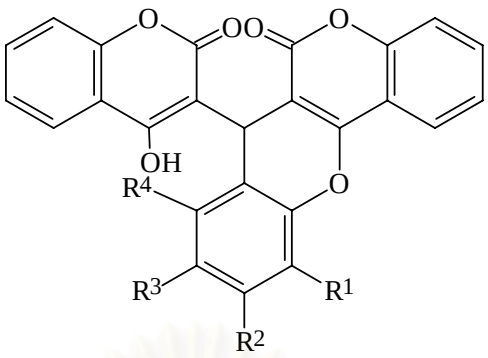
Forty-one dicoumarols including thirteen fused-rings were synthesized. Fifteen new compounds (**D18**, **D20**, **D22**, **D24**, **D25-D28**, **D32** and **D34-D39**) based upon no report of those compounds available in chemical literature can be synthesized. The comparative results of the synthetic compounds in this research are tabulated in Table 3.2 and their structures are shown below.



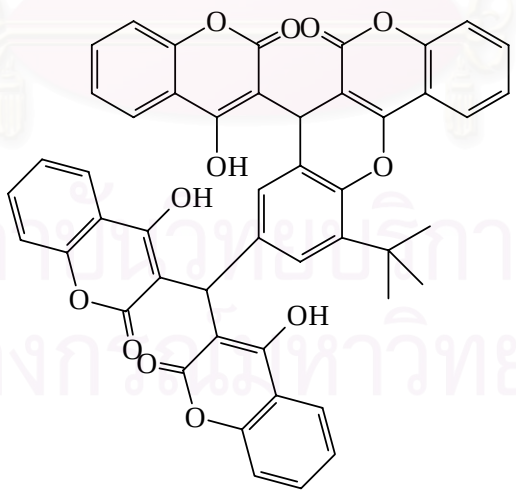
D1



Compound	R^1	R^2	R^3	R^4
D2	H	H	H	H
D3	H	H	NO_2	H
D17	H	H	H	Cl
D18	H	H	NO_2	Cl
D19	H	H	OCH_3	Cl
D20	H	-OCH_2O-		Cl
D21	H	H	H	CH_3
D22	H	H	NO_2	CH_3
D23	H	H	OCH_3	CH_3
D24	H	-OCH_2O-		CH_3
D25	H	H	H	OCH_3
D26	H	H	NO_2	OCH_3
D27	H	H	OCH_3	OCH_3
D28	H	-OCH_2O-		OCH_3



Compound	R^1	R^2	R^3	R^4
D29	H	H	H	H
D31	H	H	OCH_3	H
D32	CHO	H	OCH_3	H
D33	H	H	CH_3	H
D34	CHO	H	CH_3	H
D35	$C(CH_3)_3$	H	CH_3	H
D36	H	H	$C(CH_3)_3$	H
D37	$C(CH_3)_3$	H	$C(CH_3)_3$	H
D38	$C(CH_3)_3$	H	OCH_2CH_3	H



D39

Table 3.2 Physical properties and %yield of synthesized dicoumarols

Cpd	Physical Properties		%Yield	References
	Appearance	m.p. (°C)		
D1	white solid	287-288	40	12
D2	white crystal	232-233	93	28
D3	yellow crystal	236-237	91	29
D4	white crystal	251-252	81	30
D17	white crystal	179-180	95	31
D18	yellow crystal	263-264	39	new compound
D19	white crystal	233-234	92	31
D20	pale yellow crystal	173-174	85	new compound
D21	white crystal	235-237	73	31
D22	white powder	270-272	57	new compound
D23	pale yellow solid	200-202	62	31
D24	pale yellow solid	225-227	56	new compound
D25	white crystal	265-267	65	new compound
D26	pale yellow crystal	263-265	71	new compound
D27	pale yellow crystal	197-199	75	new compound
D28	yellow crystal	258-260	70	new compound
D29	white needle	251-253	57	11
D31	white solid	274-276	45	11
D32	yellow solid	238-240	31	new compound
D33	white solid	274-276	39	32
D34	pale yellow solid	286-288	29	new compound
D35	white crystal	271-273	30	new compound
D36	white crystal	262-263	80	new compound
D37	white crystal	246-248	59	new compound
D38	white powder	260-262	36	new compound
D39	white powder	244-246	36	new compound

3.3 Spectroscopic Data

The structures of all synthesized compounds were well characterized using various spectroscopic techniques including IR, ^{1}H-NMR, ^{13}C-NMR and MS.

Infrared Spectroscopy (IR)

Phenyl acetates

FT-IR spectra of phenyl acetates generally showed the absorption band of C-H aromatic stretching vibration at 3029-3107 cm^{-1} (w), C-H stretching vibration of CH_3 at 2925-2981 cm^{-1} (w) and the C=C ring stretching vibration at 1444-1600 cm^{-1} (s). The C=O stretching vibration of esters was observed at 1750-1778 cm^{-1} (s) and other absorption peaks belonging to C-O stretching vibration were detected at 1012-1200 cm^{-1} (s).

2-Hydroxyacetophenones

FT-IR spectra of 2-hydroxyacetophenones revealed the absorption band of O-H stretching vibration in the range of 2500-3300 cm^{-1} (br, s). The C-H stretching vibration of aromatic around 3010-3070 cm^{-1} (w) and that of C=C ring stretching at 1477-1500 cm^{-1} (s) were also found. The absorption bands corresponded to C=O stretching vibration of ketone were presented at 1650 cm^{-1} (s). Other absorption peaks of C-O stretching vibration were detected at 1010-1250 cm^{-1} (s).

4-Hydroxycoumarins

FT-IR spectra of 4-hydroxycoumarins showed the presence of O-H stretching vibration around 2450-3700 cm^{-1} (br, w). The C-H stretching vibration of aromatic was detected at 3010-3105 cm^{-1} (w), while absorption peak of C-H stretching vibration was found at 2600-2978 cm^{-1} (w). The strong absorption band at 1682-1700 cm^{-1} corresponded to the C=O stretching vibration of pyrone ring and the C=C aromatic ring stetching vibration was presented around 1480-1616 cm^{-1} (s). Other absorption peaks of C-O stretching vibration at 1038-1277 cm^{-1} (s) were detected.

2-Hydroxybenzaldehydes

FT-IR spectra of 2-hydroxybenzaldehydes displayed the absorption peak of O-H stretching vibration at 3000-3750 cm^{-1} (br, s). The C-H stretching vibration of aromatic moiety around 3020-3086 cm^{-1} (w) and that of (C=O)-H stretching at 2739-2916 cm^{-1} (w) were also detected. The strong absorption band at 1604-1682 cm^{-1} was corresponded to

the C=O stretching vibration of aldehyde, while the absorption peaks of C=C ring stretching were presented at 1466-1486 cm^{-1} (s). Other absorption peaks belonging to C-O stretching vibration were detected at 1048-1307 cm^{-1} (s).

Dicoumarols

The FT-IR absorption pattern of both substituted dicoumarols and fused-ring compounds gave common characteristic of functional groups containing in the structure. The O-H stretching vibration was presented around 2400-3750 cm^{-1} (br, w). The C-H stretching vibration of aromatic at 3020-3090 cm^{-1} (w), aliphatic C-H stretching vibration at 2603-2993 cm^{-1} (w) and C=O stretching vibration of pyrone ring at 1612-1720 cm^{-1} (s) were detected. The absorption peaks of C=C ring stretching at 1451-1593 cm^{-1} (s) and other absoption peaks of C-O stretching vibration at 1043-1300 cm^{-1} (s) were also found.

The FT-IR absorption band assignments of new compounds are tabulated in Table 3.3.

Table 3.3 FT-IR absorption band assignments of new compounds

Cpd	Wave number (cm^{-1})					
	O-H	Ar-H	C-H str.	C=O	benzo	C-O
12	2500-3300 (s)	3105 (w)	2873-2958 (w)	1685 (s)	1616 (s)	1169-1242 (s)
D18	2500-3300 (s)	3082 (w)	2723-2981 (w)	1658 (s)	1566 (s)	1119-1300 (m)
D20	2500-3300 (s)	3078 (w)	2719-2912 (w)	1674 (s)	1562 (s)	1180-1254 (s)
D22	2500-3300 (s)	3070 (w)	2731-2927 (w)	1658 (s)	1574 (s)	1088-1203 (m)
D24	2500-3300 (s)	3066 (w)	2773-2900 (w)	1658 (s)	1570 (s)	1045-1203 (m)
D25	2500-3300 (s)	3064 (w)	2845-2989 (w)	1651 (s)	1574 (s)	1092-1282 (m)
D26	2500-3300 (s)	3082 (w)	2606-2962 (w)	1658 (s)	1574 (s)	1043-1282 (m)
D27	2500-3300 (s)	3070 (w)	2607-2966 (w)	1658 (s)	1574 (s)	1084-1257 (s)
D28	2500-3300 (s)	3074 (w)	2606-2966 (w)	1655 (s)	1575 (s)	1053-1252 (s)
D32	2500-3700 (s)	3078 (w)	2607-2993 (w)	1612-1658 (s)	1566 (m)	1103-1319 (m)
D34	2500-3700 (s)	3070 (w)	2623-2985 (w)	1635-1697 (s)	1566 (m)	1080-1211 (m)
D35	2500-3700 (s)	3078 (w)	2870-2954 (w)	1651-1716 (s)	1562 (m)	1058-1207 (s)
D36	2500-3700 (s)	3070 (w)	2870-2954 (w)	1612-1712 (s)	1566 (s)	1068-1227 (m)
D37	2500-3750 (s)	3074 (w)	2870-2962 (w)	1651-1720 (s)	1562 (s)	1049-1207 (s)
D38	2500-3700 (s)	3086 (w)	2870-2970 (w)	1625-1712 (s)	1558 (s)	1065-1203 (s)
D39	2500-3750 (s)	3074 (w)	2603-2958 (w)	1625-1716 (s)	1566 (s)	1103-1215 (s)

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR

Phenyl acetates

The ^{1}H-NMR spectra of phenyl acetates generally displayed a singlet signal of methyl protons with 3H integration around 2.29-2.31 ppm and the aromatic protons 4H integration at 6.94-7.58 ppm (d, J = 8.24-8.85 Hz).

2-Hydroxyacetophenones

The ^{1}H-NMR spectra of 2-hydroxyacetophenones normally exhibited 3H integration around 2.49-2.69 ppm of methyl proton signal and the aromatic protons 3H integration at 6.38-7.90 ppm (d, J = 7.12-9.60 Hz). Another signal with 1H integration detected approximately 12.07-12.74 ppm could be assigned for a hydroxy proton.

4-Hydroxycoumarins

The ^{1}H-NMR spectra of 4-hydroxycoumarins showed an olefinic proton with 1H integration singlet signal at 5.42-5.62 ppm. The aromatic protons 3H integration were assigned as singlet and doublet with various coupling constants around 6.87-8.05 ppm following the kind of substituent. The remaining 1H integration singlet signal at 12.49-12.75 ppm was a hydroxy proton.

2-Hydroxybenzaldehydes

The ^{1}H-NMR spectra of 2-hydroxybenzaldehydes displayed aromatic protons 3H integration as singlet and doublet around 6.88-8.04 ppm and a singlet signal of an aldehydic proton with 1H integration at 9.81-10.20 ppm. The other signal with 1H integration at 10.61-12.37 ppm was a hydroxy proton.

Dicoumarols

The feature and pattern of signals in the ^{1}H-NMR spectra of substituted dicoumarols and fused-ring compounds were alike. The proton of CH-bridge was found at 5.31-6.35 ppm with 1H integration singlet signal. The aromatic protons with 2-5H integration on benzylidene ring were detected as singlet, doublet and triplet around 6.60-8.18 ppm. The overlapped 4-6H integration of H-6, H-7 and H-8 exhibited as doublet and triplet at 7.12-7.70 ppm. The singlet and broad doublet signal showed at 7.33-8.24 ppm with J = 2.75-3.05 Hz (substituted dicoumarols) and J = 7.53-8.68 Hz (fused-ring

compounds) was 2H integration of H-5. The remaining two broad singlet signals around 10.36-11.69 ppm were hydroxy protons.

The ^{1}H-NMR spectral assignments of new compounds are tabulated in Tables 3.4, 3.5 and 3.6, respectively.

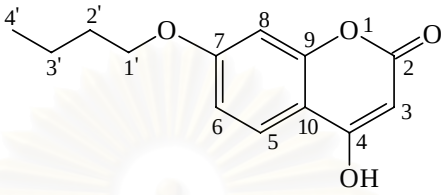
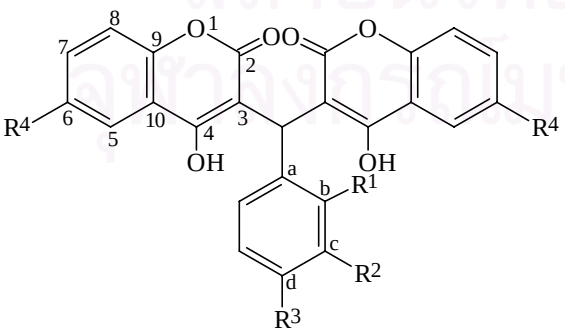


Table 3.4 ^{1}H-NMR spectral assignments of Compound **12**

Position	Chemical shift (ppm)
H-3	5.42 (s)
H-5	7.67 (dd, *J* = 6.38, 3.10 Hz)
H-6	6.87 (dd, *J* = 6.46, 2.36 Hz)
H-8	6.91 (d, *J* = 1.93 Hz)
H-1′	4.03 (t, *J* = 6.43 Hz)
H-2′	1.71 (quint, *J* = 6.33 Hz)
H-3′	1.41 (hext, *J* = 7.27 Hz)
H-4′	0.91 (t, *J* = 7.22 Hz)



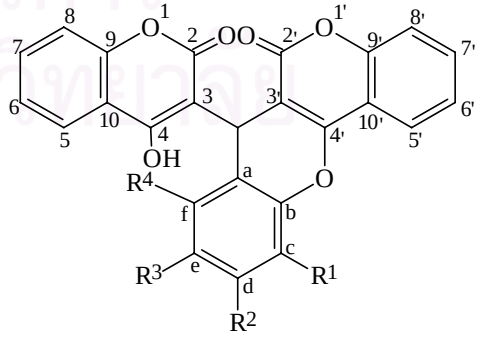


Table 3.5 ^{1}H-NMR spectral assignments of new dicoumarols

Cpd	R				chemical shift (ppm)											
	R^1	R^2	R^3	R^4	CH-Ar	H-5	H-6	H-7	H-8	OH	H-b	H-c	H-d	H-e	H-f	H-R
D18	H	H	NO_2	Cl	6.34 (s)	7.76 (d)	-	7.55 (dd)	7.33 (d)	*	7.38 (dd)	8.05 (d)	-	-	-	-
D20	H	-OCH_2O-		Cl	5.95 (s)	7.95 (s) 8.00 (s)	-	7.55 (dd)	7.33 (d)	11.19 (s) 11.47 (s)	6.60 (d)	-	-	6.72 (d)	6.61- 6.62 (m)	5.93 (s)
D22	H	H	NO_2	CH_3	6.08 (s)	7.77 (s) 7.85 (s)	-	7.37 (dd)	7.31 (d)	11.36 (s) 11.58 (s)	7.44 (d)	8.16 (d)	-	-	-	2.43 (s), 2.46 (s)
D24	H	-OCH_2O-		CH_3	5.97 (s)	7.76 (s) 7.81 (s)	-	7.40 (dd)	7.26 (d)	11.28 (s) 11.62 (s)	6.63 (d)	-	-	6.70 (d)	6.64- 6.65 (m)	2.41 (s), 2.43 (s), 5.91 (s)
D25	H	H	H	OCH_3	6.35 (s)	7.35 (d)	-	7.16 (dd)	7.30 (d)	*	7.12 (d)	7.21 (t)	7.13 (t)	-	-	3.79 (s)
D26	H	H	NO_2	OCH_3	6.09 (s)	7.34 (d) 7.43 (d)	-	7.20 (dd)	7.33 (d)	11.49 (s) 11.69 (s)	7.38 (d)	8.16 (d)	-	-	-	3.85 (s), 3.88 (s)
D27	H	H	OCH_3	OCH_3	6.02 (s)	7.36 (s) 7.42 (s)	-	7.16 (dd)	7.30 (d)	11.41 (s) 11.60 (s)	7.09 (dd)	6.83 (d)	-	-	-	3.77 (s), 3.83 (s), 3.87 (s)
D28	H	-OCH_2O-		OCH_3	6.21 (s)	7.33 (d)	-	7.13 (dd)	7.26 (d)	*	6.62 (s)	-	-	6.72 (d)	6.56 (dd)	3.78 (s), 5.92 (s)
D32	CHO	H	OCH_3	H	6.23 (s)	7.86 (dd)	7.28 (t)	7.53 (dt)	7.26 (d)	*	-	-	7.12 (d)	-	7.04 (d)	3.69 (s), 9.92 (s)
D34	CHO	H	CH_3	H	5.73 (s)	8.03 (br) 8.24 (dd)	7.46(t) 7.49 (dt)	7.60 (dt) 7.70 (dt)	7.32 (d) 7.36 (br)	*	-	-	7.55 (d)	-	7.29 (d)	2.27 (s), 10.65 (s)
D35	$C(CH_3)_3$	H	CH_3	H	5.31 (s)	8.01 (dd) 8.16 (dd)	7.27 (dt) 7.45 (dt)	7.46 (dt) 7.61 (dt)	7.15 (d) 7.40 (d)	10.36 (s)	-	-	7.07 (d)	-	6.77 (d)	1.60 (s), 2.23 (s)
D36	H	H	$C(CH_3)_3$	H	5.71 (s)	8.07 (dd)	7.45 (t)	7.57 (dt) 7.67 (dt)	7.25 (d) 7.42 (d)	*	-	7.30 (d)	7.33 (dd)	-	7.20 (s)	1.18 (s)
D37	$C(CH_3)_3$	H	$C(CH_3)_3$	H	5.70 (s)	8.02 (br) 8.05 (dd)	7.34 (t) 7.54 (dt)	7.58 (dt) 7.70 (dt)	7.28 (d) 7.46 (d)	*	-	-	7.25 (d)	-	7.10 (d)	1.20 (s), 1.55 (s)
D38	$C(CH_3)_3$	H	OCH_2CH_3	H	5.32 (s)	8.00 (dd) 8.14 (dd)	7.27 (t) 7.45 (t)	7.46 (dt) 7.62 (dt)	7.16 (d) 7.41 (d)	10.41 (s)	-	-	6.84 (d)	-	6.43 (d)	1.32 (t), 1.58 (s), 3.90 (quart)

* : not assigned

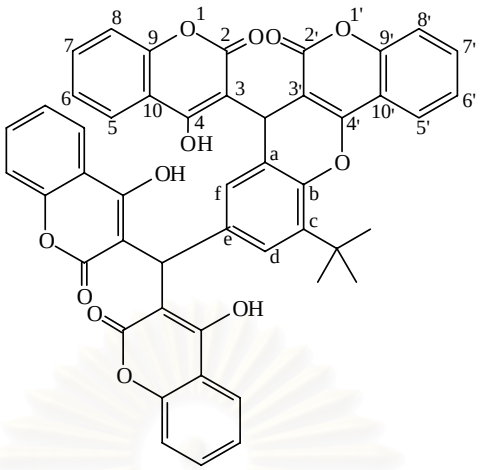


Table 3.6 ^{1}H-NMR spectral assignments of Compound **D39**

Position	Chemical shift (ppm)
H-5, 5′	7.51 (br), 7.78 (br), 7.87 (dd, *J* = 7.94, 1.53 Hz), 8.02 (dd, *J* = 7.94, 1.22 Hz)
H-6, 6′	7.12 (t, *J* = 7.93 Hz), 7.28 (t, *J* = 7.93 Hz), 7.29 (t, *J* = 7.94 Hz), 7.31 (t, *J* = 6.86 Hz)
H-7, 7′	7.52 (t, *J* = 7.93 Hz), 7.58 (dt, *J* = 8.55, 2.44 Hz), 7.58 (dt, *J* = 6.87, 2.74 Hz), 7.68 (dt, *J* = 7.79, 1.53 Hz)
H-8, 8′	7.27 (d, *J* = 7.94 Hz), 7.35 (d, *J* = 8.24 Hz), 7.44 (d, *J* = 8.24 Hz), 7.49 (d, *J* = 7.33 Hz)
C<u>H</u>-Ar	5.58 (s), 6.24 (s)
H-d	7.04 (d, *J* = 1.52 Hz)
H-f	6.88 (s)
C(C<u>H</u>$_3$)$_3$	1.42 (s)

^{13}C-NMR

Phenyl acetates

The ^{13}C-NMR spectra of phenyl acetates normally exhibited a carbonyl carbon of ester at 169.2-169.5 ppm and a methyl carbon adjacent to an ester functional group at 20.9-21.1 ppm. The set of signal detected for aromatic carbons as C-1 (1C), C-2 (2C), C-3 (2C) and C-4 (1C) showed in the range of 148.5-150.1, 121.2-122.9, 128.1-129.8 and 131.2-139.0 ppm, respectively.

2-Hydroxyacetophenones

The ^{13}C-NMR spectra of 2-hydroxyacetophenones displayed a methyl carbon at 25.9-26.7 ppm and a carbonyl carbon of ketone at 202.4-204.6 ppm. The remaining 6C signals revealed at 119.4-129.0, 160.3-161.8, 118.2-120.1, 135.4-137.5, 123.5-132.4 and 126.6-130.4 ppm were aromatic carbons as C-1, C-2, C-3, C-4, C-5 and C-6 (1C each), respectively.

4-Hydroxycoumarins

The carbons of benzopyrone ring as C-2, C-3, C-4, C-5, C-6 ,C-7, C-8, C-9 and C-10 (1C each) were observed at 161.3-163.1, 88.4-91.7, 164.4-167.3, 104.9-157.3, 106.0-147.8, 120.3-162.3, 100.5-118.7, 151.8-155.4 and 105.0-124.3 ppm, respectively.

2-Hydroxybenzaldehydes

The ^{13}C-NMR spectra of 2-hydroxybenzaldehydes showed a carbonyl carbon of aldehyde at 189.9-197.1 ppm. The aromatic carbons as C-1, C-2, C-3, C-4, C-5 and C-6 (1C each) were detected at 120.0-123.5, 152.6-166.1, 117.2-139.9, 122.5-138.0, 128.1-158.0 and 115.2-133.8 ppm, respectively.

Dicoumarols

The ^{13}C-NMR spectra of substituted dicoumarols revealed the peak of CH-bridge at 28.6-36.8 ppm. The aromatic carbons of 2 benzopyrone ring (18C) as C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9 and C-10 (2C each) were assigned around 152.0-166.4, 99.2-116.5, 158.4-169.4, 105.0-132.4, 122.2-156.7, 107.4-133.8, 116.2-123.3, 144.9-153.1 and 114.2-120.8 ppm, respectively. The remaining 6C of benzylidene ring exhibited in the range of 107.0-158.6 ppm. For the fused-ring compounds, they were found to be similar to those of substituted dicoumarols.

The ^{13}C-NMR spectral assignments of new compounds are presented in Tables 3.7, 3.8 and 3.9, respectively.

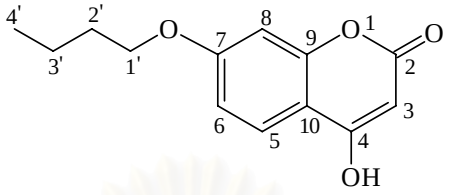
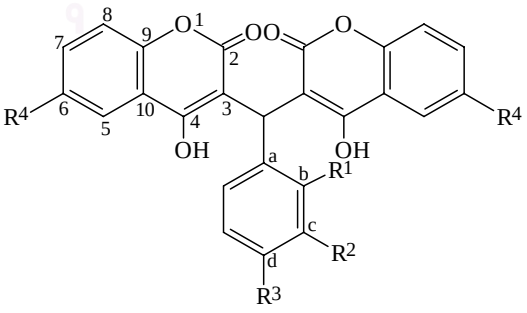


Table 3.7 ^{13}C-NMR spectral assignments of Compound **12**

Position	Chemical shift (ppm)
C-2	162.3
C-3	88.4
C-4	166.0
C-5	124.0
C-6	112.1
C-7	162.3
C-8	100.9
C-9	155.4
C-10	108.7
C-1′	67.9
C-2′	30.5
C-3′	18.6
C-4′	13.6



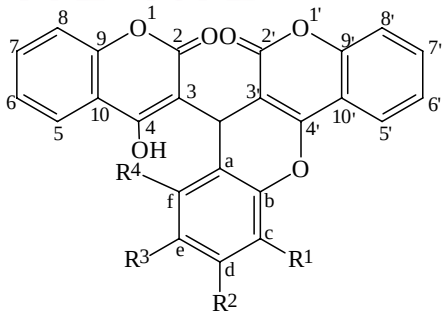


Table 3.8 ^{13}C-NMR spectral assignments of new dicoumarols

Cpd	R				Chemical shift (ppm)																
	R^1	R^2	R^3	R^4	CH-Ar	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-a	C-b	C-c	C-d	C-e	C-f	C(R)
D18	H	H	NO_2	Cl	36.8	163.8	103.5	166.1	123.2	127.4	127.9	117.8	151.1	120.8	145.5	131.0	123.1	150.3	-	-	-
D20	H	-OCH_2O-		Cl	36.0	163.5	104.6	166.3	123.9	128.2	133.0	118.1	150.5	117.5	130.6	108.3	148.3	146.6	107.0	119.5	101.3
						164.6	106.3	168.8					150.8	117.9							
D22	H	H	NO_2	CH_3	36.6	164.9	103.3	167.1	124.0	135.1	127.6	116.5	150.6	116.0	143.6	134.4	123.8	146.9	-	-	21.0
						166.4	104.8	169.2		135.2		116.6	150.9	116.4							
D24	H	-OCH_2O-		CH_3	35.9	164.5	103.9	166.8	123.9	134.7	133.8	116.3	150.4	116.0	129.1	108.1	148.1	146.3	107.2	119.5	21.0, 101.1
						165.7	105.6	169.3					150.7	116.5							
D25	H	H	H	OCH_3	36.0	164.9	104.2	165.4	105.6	155.3	117.2	119.7	146.6	118.6	140.3	128.0	126.7	125.4	-	-	55.6
D26	H	H	NO_2	OCH_3	36.6	164.6	103.4	167.0	105.1	156.7	117.9	122.1	147.1	116.6	143.4	127.5	123.8	146.8	-	-	55.9, 56.0
						166.2	104.9	169.1	105.2		118.0	122.2		117.1							
D27	H	H	OCH_3	OCH_3	35.5	164.3	104.3	166.8	105.0	156.5	113.9	121.5	146.7	116.8	127.0	127.5	117.8	158.3	-	-	55.2, 55.8,
						165.4	105.9	169.3	105.1			121.6	147.0	117.3							55.9
D28	H	-OCH_2O-		OCH_3	35.9	164.7	104.3	165.9	105.6	155.1	107.4	119.4	144.9	119.1	134.8	117.0	147.1	146.6	107.6	119.5	55.5, 100.6
D32	CHO	H	OCH_3	H	32.6	163.7	103.6	165.4	123.8	123.4	131.4	118.5	151.6	120.1	131.9	152.2	125.3	110.8	153.8	115.8	55.3, 196.6
D34	CHO	H	CH_3	H	28.4	160.3	113.7	161.2	124.8	124.1	132.8	123.1	152.0	116.2	128.4	155.7	123.5	132.4	134.7	135.2	20.1, 189.2
							116.5					123.3	152.3	116.4							
D35	$C(CH_3)_3$	H	CH_3	H	34.9	160.9	99.8	166.1	125.1	123.7	131.8	116.2	152.1	115.0	121.1	147.9	136.7	123.3	134.3	127.1	21.0, 30.1,
						161.1	109.1	158.4	127.0	124.3	132.5	117.2	153.0	116.9							30.2
D36	H	H	$C(CH_3)_3$	H	34.0	152.0	113.9	160.4	132.2	124.5	132.4	122.6	147.2	116.1	121.4	156.5	116.5	123.9	124.7	125.4	29.0, 31.0
						152.2	115.8						147.7	116.2							
D37	$C(CH_3)_3$	H	$C(CH_3)_3$	H	34.8	152.1	107.0	165.4	132.4	123.2	124.8	122.3	146.0	114.2	123.9	156.3	132.2	116.9	135.9	122.5	29.2, 30.1,
						152.2	108.0	160.5					146.7	116.2							31.1, 34.2
D38	$C(CH_3)_3$	H	OCH_2CH_3	H	35.1	161.0	99.2	166.3	124.3	123.3	131.8	116.2	152.1	115.0	122.1	144.1	138.6	110.5	155.6	113.8	14.8, 30.1,
						161.1	108.9	158.5	125.1	123.8	132.5	117.2	153.0	116.9							30.7, 63.7

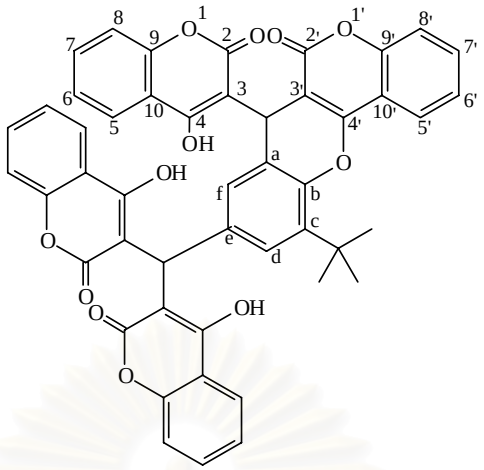


Table 3.9 ^{13}C-NMR spectral assignments of Compound **D39**

Position	Chemical shift (ppm)
C-2, 2′	164.6, 164.9
C-3, 3′	103.4, 104.1
C-4, 4′	165.8, 165.3, 160.5
C-5, 5′	123.7, 123.8, 123.9, 124.0
C-6, 6′	123.5, 123.7, 124.8
C-7, 7′	131.7, 131.9, 132.4
C-8, 8′	114.2, 116.0
C-9, 9′	152.0, 152.1
C-10, 10′	115.7, 116.8, 117.8, 118.1
<u>C</u>H-Ar	34.5
C-a	125.4
C-b	152.2
C-c	135.7
C-d	122.3
C-e	135.3
C-f	132.0
$\underline{C}(CH_3)_3$	35.7
$C(\underline{C}H_3)_3$	29.9

Mass Spectroscopy (MS)

The mass spectrum was used to confirm the structures of new compounds. The mass spectra of these synthesized compounds showed the molecular ion (M^+) with small relative intensity or sometimes it could not be observed as found in Compounds **D18**, **D27**, **D28**, **D32** and **D39**. The MS spectral assignments for these new compounds are presented in Table 3.10.

Table 3.10 Mass spectral assignments of new compounds

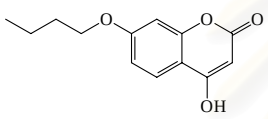
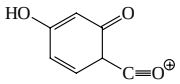
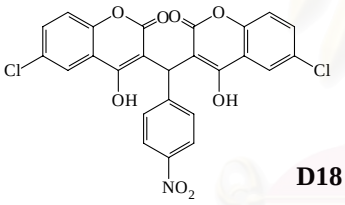
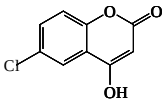
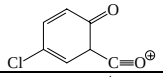
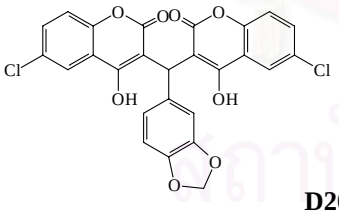
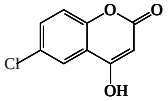
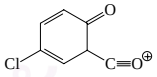
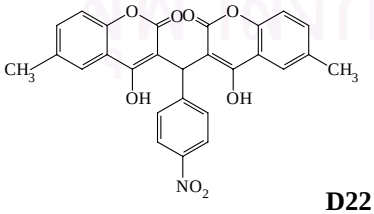
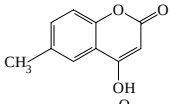
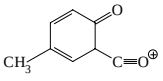
Cpd	Peak (m/z)	Assignment
 **12**	234 178 150 136	M^+ M^+-C_4H_8 M^+- C_4H_8-CO 
 **D18**	329 196 154	M^+-$C_9H_5ClO_3$  
 **D20**	524 328 196 154	M^+ M^+-$C_9H_5ClO_3$  
 **D22**	485 309 176 134	M^+ M^+-$C_{10}H_8O_3$  

Table 3.10 (cont.)

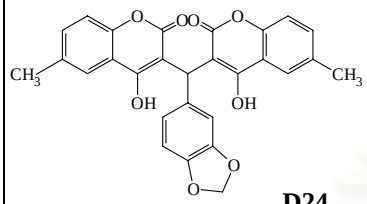
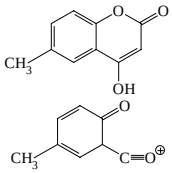
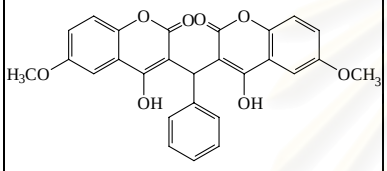
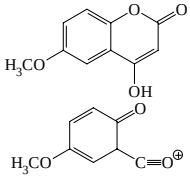
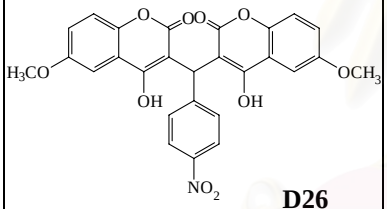
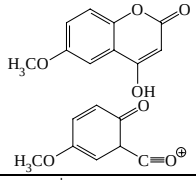
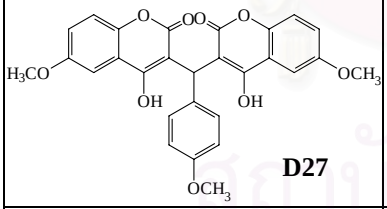
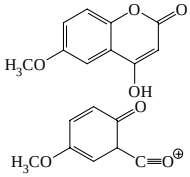
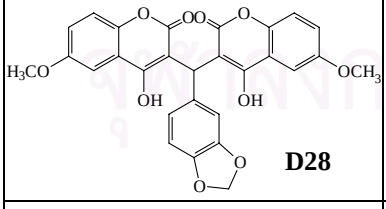
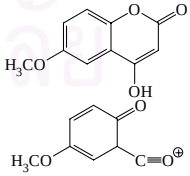
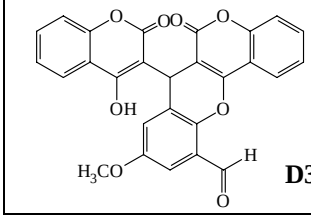
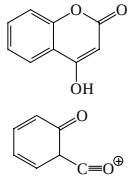
Cpd	Peak (m/z)	Assignment
 **D24**	484 308 176 134	M^+ M^+-$C_{10}H_8O_3$ 
 **D25**	472 280 192 150	M^+ M^+-$C_{10}H_8O_4$ 
 **D26**	517 325 192 150	M^+ M^+-$C_{10}H_8O_4$ 
 **D27**	310 192 150	M^+-$C_{10}H_8O_4$ 
 **D28**	324 192 150	M^+-$C_{10}H_8O_4$ 
 **D32**	307 162 120	M^+-$C_9H_5O_3$ 

Table 3.10 (cont.)

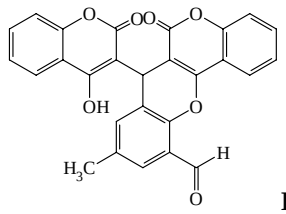
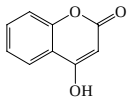
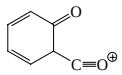
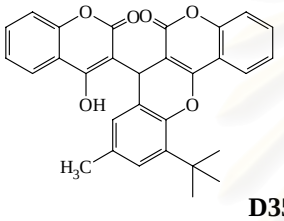
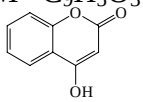
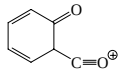
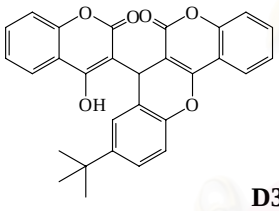
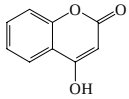
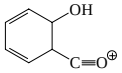
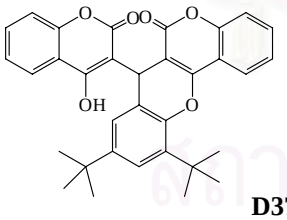
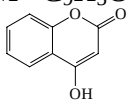
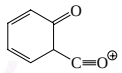
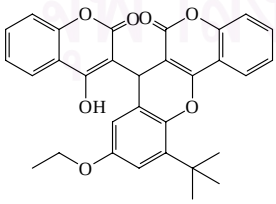
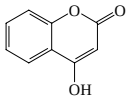
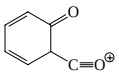
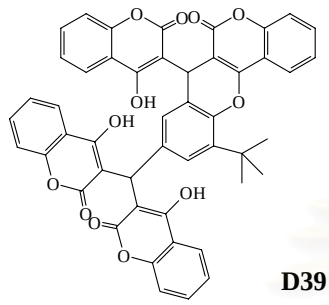
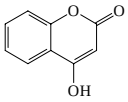
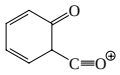
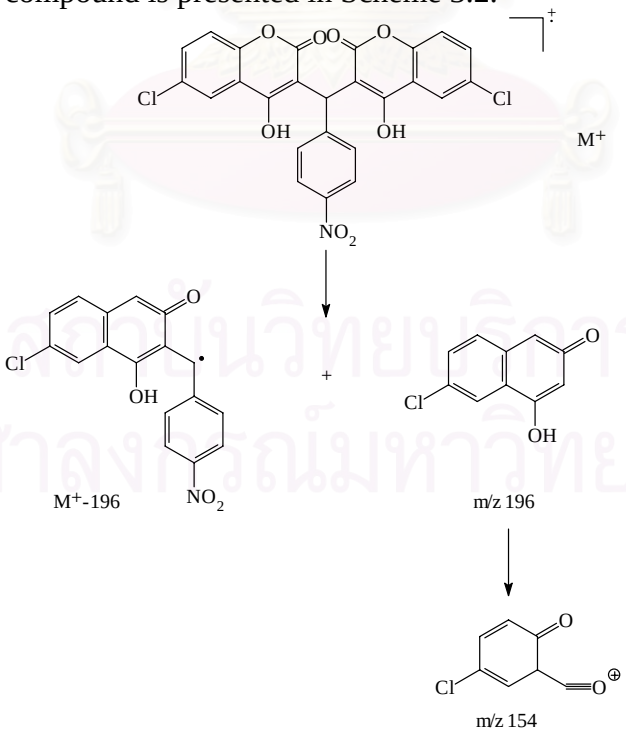
Cpd	Peak (m/z)	Assignment
 **D34**	452 291 162 120	M^+ M^+-$C_9H_5O_3$  
 **D35**	480 319 162 120	M^+ M^+-$C_9H_5O_3$  
 **D36**	466 305 162 121	M^+ M^+-$C_9H_5O_3$  
 **D37**	522 361 162 120	M^+ M^+-$C_9H_5O_3$  
 **D38**	510 349 162 120	M^+ M^+-$C_9H_5O_3$  

Table 3.10 (cont.)

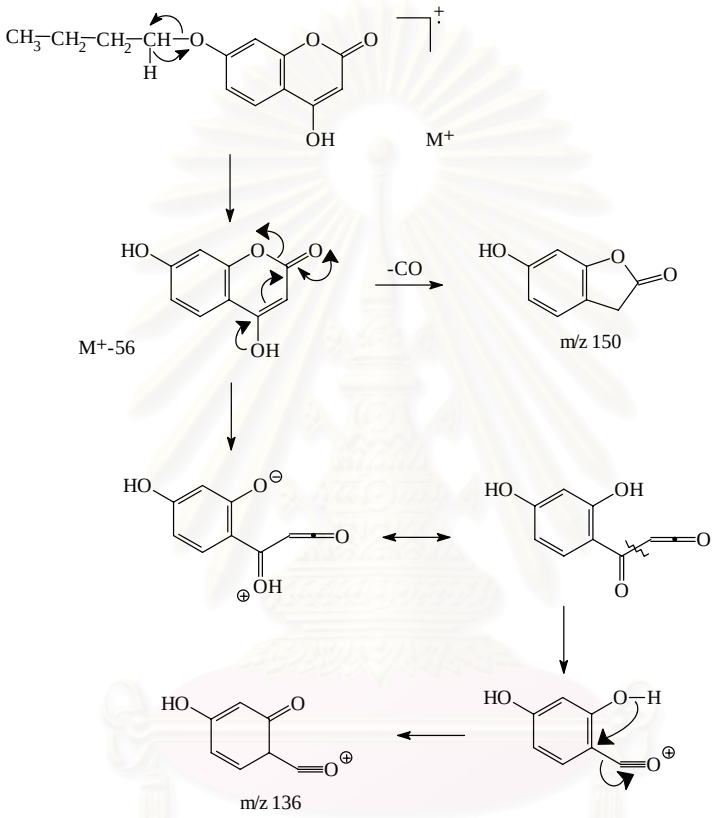
Cpd	Peak (m/z)	Assignment
 **D39**	314 162 120	M^+-3($C_9H_5O_3$)  

Both of substituted dicoumarols and fused-ring compounds provided similarly fragmentation pattern. They showed a molecular ion [M^+] and a peak formed by loss of a monomer. This fragmentation pattern gave a peak of 4-hydroxycoumarin derivative which could be confirmed by a characteristic peak owing to the lost of C_2H_2O from the pyrone ring. The proposed fragmentation pattern of dicoumarols (**D18**) as a selected representative compound is presented in Scheme 3.2.



Scheme 3.2 The proposed mass fragmentation pattern of Compound **D18**

In the case of Compound **12**, its mass spectrum is little difference from that of dicoumarols. Molecular ion peak [M^+ 234] was found as a base peak. It lost butyl to form m/z 178 of [M^+-C_4H_8] and then CO group to generate m/z 150. The other one is m/z 136 which formed by loss of C_2H_2O. The possible fragmentation pattern of dicoumarols **12** is presented in Scheme 3.3.



Scheme 3.3 The possible mass fragmentation pattern of Compound **12**

3.4 Biological Activities

Almost all investigations have reported that both 4-hydroxycoumarins and dicoumarols have been found to possess anticoagulant activity.[1,36] However, very recently they have also been recorded to reveal other activities, for example, they exhibited pronounced antiviral properties against HIV-1 protease and integrase.[37,38,39,40] Moreover, certain 4-hydroxycoumarin derivatives were addressed to possess antibacterial activity.[41]

3.4.1 Biological Activity of 4-Hydroxycoumarins

3.4.1.1 Brine Shrimp Cytotoxic Lethality Test against *Artemia salina* Leach.

Twelve synthesized 4-hydroxycoumarins were subjected to brine shrimp lethality test. The results are presented as shown in Table 3.11. 4-Hydroxycoumarin **(1)** was used as a reference compound. Based upon the results obtained, two prepared coumarins (**4** and **9**) displayed better activity than **(1)**, while other coumarins exhibited almost the same or lower activities

.

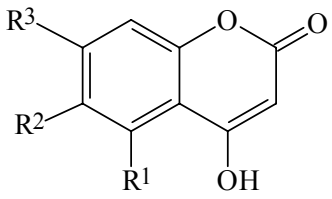


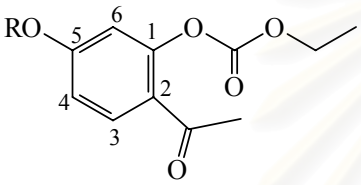
Table 3.11 LC_{50} value at 24 hours of tested 4-hydroxycoumarins

Cpd	R^1	R^2	R^3	LC_{50} (μg/mL)	Bioactivity
1	H	H	H	37.48	Medium
2	H	CH_3	H	37.26	Medium
3	OCH_3	H	H	37.67	Medium
4	H	OCH_3	H	19.21	Medium
5	H	H	OCH_3	39.55	Medium
6	H	Cl	H	43.73	Medium
7	H	Br	H	37.31	Medium
8	H	Br	OCH_3	85.30	Medium
9	H	CH_2CH_3	H	27.73	Medium
10	H	Ph	H	81.30	Medium
11	H	H	OCH_2Ph	273.14	Low
12	H	H	$O(CH_2)_3CH_3$	530.97	Low

Note: $LC_{50} < 10$ μg/mL = High activity
$LC_{50} < 100$ μg/mL = Medium activity
$LC_{50} < 1000$ μg/mL = Low activity
$LC_{50} > 1000$ μg/mL = Inactive

The data from brine shrimp lethality results indicated that the position and type of substituents were major parameters to influence this activity. For instance, in the case of the compounds containing methoxy substituents at different position, it was observed that a methoxy group at position 6 increased the activity whereas a methoxy group at positions 5 or 7 showed the same level of activity comparing with a reference compound

(Compound **1**). In addition, a variety of substituents at position 6 or 7 affected the activity. Among various substituents at position 6 of 4-hydroxycoumarins, a methoxy substituent generally gave a better result than other substituents. In a case of alkoxy groups at position 7, three compounds (**5**, **11** and **12**) markedly decreased the brine shrimp lethality, particularly butyloxy and benzyloxy substituents. On the contrary, non-cyclized compounds (**12c-15c**) containing alkoxy substituents at position 5 exhibited higher activities than cyclized compounds. The LC_{50} value of non-cyclized compounds is shown below.



Cpd	R	LC_{50} (μg/mL)	Bioactivity
12c	$(CH_2)_3CH_3$	69.90	Medium
13c	$(CH_2)_5CH_3$	5.76	High
14c	$(CH_2)_7CH_3$	3.02	High
15c	$(CH_2)_{11}CH_3$	3.58	High

3.4.1.2 Weed Growth Inhibition against *Mimosa pigra* Linn.

In this bioassay, *Mimosa pigra* (giant sensitive plant) which is a medium tree and has been recognized as a weed in both water and land was selected to test by using the chemical method. Twelve synthesized 4-hydroxycoumarins were investigated to weed growth inhibition. The results are presented as shown in Table 3.12. The results of a representative compound (**3**) were exemplified as shown in Fig 3.1. The concentration of tested 4-hydroxycoumarins is in the range of 1-1000 ppm. The % inhibition of root is thought to be more essential for considering than that of % shoot inhibition. This is due to the fact that the roots are directly contacted to the tested chemical in cellulose. Percent root inhibition at 100 ppm was chosen to be mainly contemplated. Because low root inhibition always exhibited by substance with low concentration (1-10 ppm) and substance with high concentration (1000-10000 ppm) always ceased herb growth at high level. Therefore, the interpretations of these results are intensively concentrated at the first view on the tendency of inhibition and % root inhibition at dose level 100 ppm.

Table 3.12 The results of weed growth inhibition of 4-hydroxycoumarins against *M. pigra*

Cpd	% Growing inhibition at (ppm)							
	Root				Shoot			
	1	10	100	1000	1	10	100	1000
1	-20.91	-10.49	14.53	87.49	-4.65	-0.03	13.82	90.77
2	-18.82	-22.99	-10.49	41.63	-3.11	-6.19	16.90	56.91
3	-8.40	4.11	62.48	74.98	-1.57	-1.57	27.67	61.53
4	-6.32	-18.82	-6.32	20.78	-6.19	-4.65	7.66	30.75
5	-22.99	-14.65	-12.57	27.04	-7.73	-6.19	1.51	36.90
6	-8.40	6.19	37.46	77.07	-30.81	19.98	29.21	75.38
7	-18.82	6.19	4.11	52.05	-7.73	13.82	9.20	72.30
8	-6.32	-18.82	-16.74	-8.40	-15.42	-4.65	3.05	27.67
9	4.11	29.12	10.36	66.65	-6.19	13.82	7.66	70.76
10	14.53	4.11	4.11	60.39	-9.26	9.20	7.66	53.83
11	-47.22	13.89	-5.56	61.11	-21.50	13.78	-9.74	49.05
12	-18.22	8.28	18.70	64.56	1.51	18.44	39.98	78.45
H1	-	45.67	80.39	80.71	-	40.01	18.76	10.01
H2	-	-1.92	52.74	81.67	-	1.26	46.26	52.51

Note: Herbicides

H1 = Mets, active ingredient: methyl-2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonylamino]-sulfonyl]-benzoate

H2 = Hexaz, active ingredient: 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione

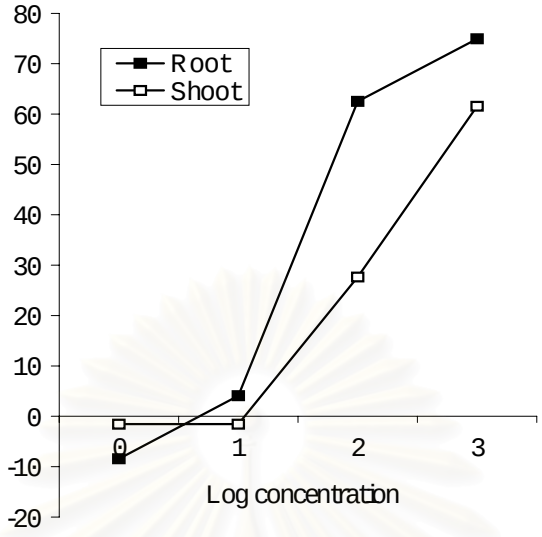


Fig 3.1 Percent root and shoot growing inhibition of Compound **3** against *M. pigra*

From the obtained data, it was observed that five prepared 4-hydroxycoumarins (**1**, **3**, **5**, **6** and **12**) gave good tendency of inhibition. Among them, only **5** displayed lower activity than **1** (a reference compound), whereas **3** exhibited the highest activity. Considering the type of substituent, it was found that a methoxy group showed medium activity (62 %), whereas chloro atom and butyloxy group had a little effect on % antigrowth. In addition, the position of substituents also affected the growth inhibition. Comparing the methoxy group at positions 5, 6 and 7, it could obviously be seen that a methoxy group at position 5 enhanced the activity, while the other positions (C-6, C-7) decreased the inhibition activity.

3.4.2 Biological Activity of Dicoumarols

3.4.2.1 Brine Shrimp Cytotoxic Lethality Test against *Artemia salina* Leach.

A recent research described that 4-hydroxycoumarins with chloro and methyl substituents at C-6 or a methoxy group at C-5 displayed high antifeedant activity and weed growing inhibition.[17] In the year of 2000, dicoumarols with various substituents on benzylidene ring were manipulated and examined on brine shrimp lethality test. It was reported that most of them exhibited medium activity.[18] Therefore, to extend the work in

this trail this research was focussed on investigation of various substituents at position 6 on a benzopyrone ring of dicoumarols on this activity.

Sixteen synthesized dicoumarols were subjected to brine shrimp lethality test. The results are summarized in Table 3.13. These dicoumarols were divided into four groups. Considering compounds **D2**, **D3**, **D15** and **D16** as the reference compounds of each group, all dicoumarols with chloro, methyl or methoxy substituents at position 6 on the benzopyrone ring provided the decreasing of this activity.

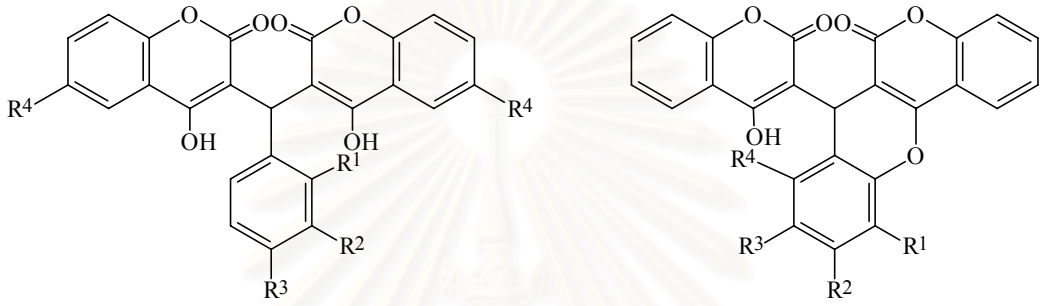


Table 3.13 LC_{50} value at 24 hours of tested dicoumarols

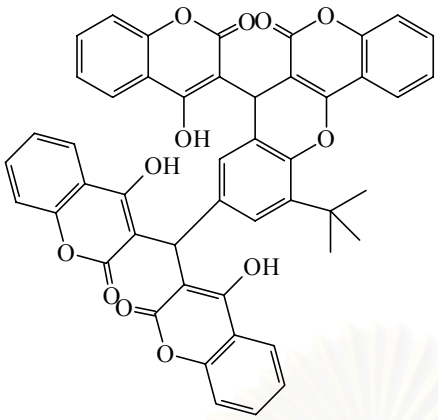
Group	Cpd	R^1	R^2	R^3	R^4	LC_{50} (μg/mL)	Bioactivity
I	**D2**	H	H	H	H	13.18	Medium
	D17	H	H	H	Cl	385.67	Low
	D21	H	H	H	CH_3	761.66	Low
	D25	H	H	H	OCH_3	50.54	Medium
II	**D3**	H	H	NO_2	H	31.62	Medium
	D18	H	H	NO_2	Cl	260.02	Low
	D22	H	H	NO_2	CH_3	1028.59	Inactive
	D26	H	H	NO_2	OCH_3	91.60	Medium
III	**D15**	H	H	OCH_3	H	27.62	Medium
	D19	H	H	OCH_3	Cl	31.03	Medium
	D23	H	H	OCH_3	CH_3	368.66	Low
	D27	H	H	OCH_3	OCH_3	47.15	Medium
IV	**D16**	H	-OCH_2O-		H	118.20	Low
	D20	H	-OCH_2O-		Cl	784.13	Low
	D24	H	-OCH_2O-		CH_3	922.85	Low
	D28	H	-OCH_2O-		OCH_3	99.84	Medium

Among a variety of substituents at position 6 on a benzopyrone ring studied, a methyl group showed the lowest activity, less than chloro and methoxy groups, respectively except for **D19** and **D27** in group III.

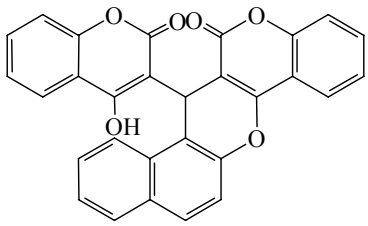
Furthermore, thirteen fused-ring dicoumarols were prepared and examined for SAR study. The data of brine shrimp lethality result are tabulated in Table 3.14. A variation of substituents on a benzopyran ring was obsearved using **D29** as reference. It was found that three fused-ring compounds (**D37**, **D39** and **D40**) exhibited better activities than **D29**, whereas other fused-ring compounds displayed lower activities. In the case of **D35**, the activity was found in the same level as **D29**.

Table 3.14 LC_{50} value at 24 hours of fused-ring compounds

Cpd	R^1	R^2	R^3	R^4	LC_{50} (μg/mL)	Bioactivity
D29	H	H	H	H	21.23	Medium
D30	OCH_3	H	H	H	40.54	Medium
D31	H	H	OCH_3	H	81.26	Medium
D32	CHO	H	OCH_3	H	516.52	Low
D33	H	H	CH_3	H	42.00	Medium
D34	CHO	H	CH_3	H	1182.16	Inactive
D35	$C(CH_3)_3$	H	CH_3	H	21.17	Medium
D36	H	H	$C(CH_3)_3$	H	27.73	Medium
D37	$C(CH_3)_3$	H	$C(CH_3)_3$	H	9.00	High
D38	$C(CH_3)_3$	H	OCH_2CH_3	H	45.88	Medium
D40	H	H	H	Cl	12.79	Medium



D39 (LC_{50} = 10.59 μg/mL)



D41 (LC_{50} = 207.40 μg/mL)

Considering of the monosubstituents at position 3 on a benzopyran ring, it was noted that tertiary butyl group displayed the highest activity, more than methyl and methoxy groups, respectively. For fused-ring compounds with disubstituents, carboxaldehyde group declined the activities of **D32** and **D34** while tertiary butyl group provided the enhancing activity of **D35**. In the case of **D37**, two tertiary butyl groups at position 3 and 5 showed the best activity.

3.4.2.2 Static Acute Toxicity Bioassay of Tilapia, *Oreochromis niloticus*

Although the literatures contain an enormous number of dicoumarols and their derivatives that are claimed to have beneficial anticoagulant, acute toxicity test of dicoumarols has never been reported in literatures before. This bioassay was developed and applied for primary biological screening. The selected representative of small veterbrates in this test is *Oreochromis niloticus*. Seventeen synthesized dicoumarols were subjected to acute toxicity test. The LC_{50} value is revealed in Table 3.15. The percent mortality of *O. niloticus* of Compound **D3** at 24, 48, 72 and 96 hours is presented as an instance shown in Figure 3.2.

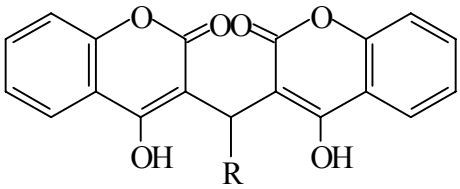


Table 3.15 LC_{50} value at 96 hours of tested dicoumarols

Cpd	R	LC_{50} (μg/mL)	Level of activity
D1	H	0.23	+++++
D2	C_6H_5	4.04	++++
D5	4-FC_6H_4	4.59	++++
D4	4-ClC_6H_4	16.89	+++
D8	4-BrC_6H_4	4.60	++++
D7	3-BrC_6H_4	7.55	++++
D6	2-BrC_6H_4	35.88	+++
D3	4-$(NO_2)C_6H_4$	0.24	+++++
D15	4-$(OCH_3)C_6H_4$	872.54	+
D14	3-$(OCH_3)C_6H_4$	103.82	++
D13	2-$(OCH_3)C_6H_4$	8.10	++++
D9	4-$(CH_3)C_6H_4$	133.65	++
D10	4-(i-Pr)C_6H_4	528.90	+
D11	4-(t-Bu)C_6H_4	103.07	++
D12	4-$CF_3(C_6H_4)$	6.19	++++
D16	3,4-methylenedioxybenzyl	-	-
D29	C_6H_4 (fuse)	8.04	++++
1,4-naphthoquinone		0.11	+++++

Note: Level of activity

LC_{50} = 0.1-1	μg/mL	+++++	LC_{50} = 101-500	μg/mL	++
LC_{50} = 1-10	μg/mL	++++	LC_{50} = 501-1000	μg/mL	+
LC_{50} = 10-100	μg/mL	+++	LC_{50} > 1000	μg/mL	-

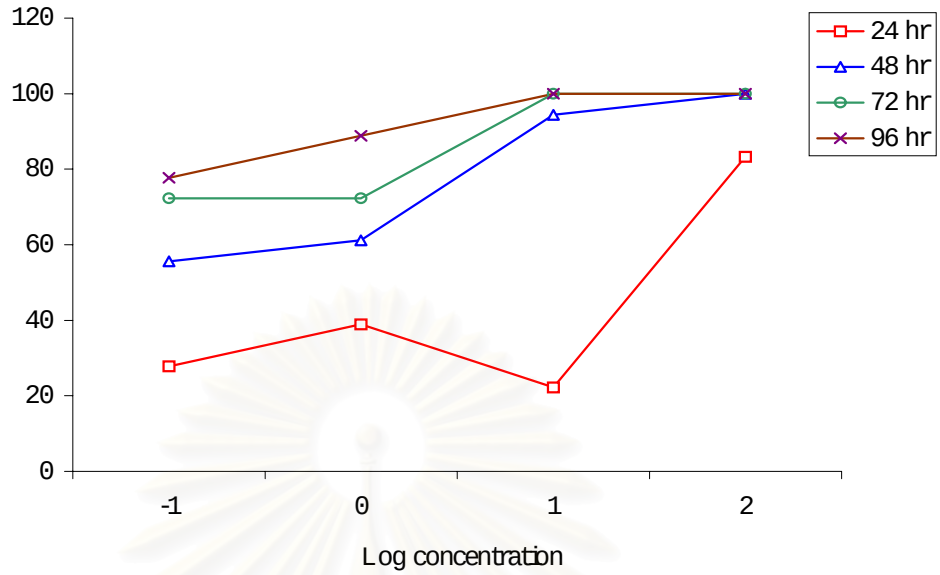


Fig 3.2 Percent mortality of *O. niloticus* of Compound **D3** at 24, 48, 72 and 96 hrs

From the view of comparison of various substituents at position 4 on a benzylidene ring of dicoumarols using Compound **D2** (R=C_6H_5) as a reference, it was found that various substituents at 4-position reduced activity except for a nitro group which showed higher activity than the parent compound.

All synthesized dicoumarols could be classified into two types according to substituents as follows: 1) electron withdrawing groups (F, Cl, Br and NO_2) and 2) electron donating groups (OCH_3, CH_3, *i*-Pr, *t*-Bu and 3,4-methylenedioxy). Referring to the obtained data, it was observed that most of dicoumarols bearing electron withdrawing substituents displayed higher activities than those containing electron donating substituents. Considering of halogen and nitro groups, the activity was found to be variable in the range of high activity around 0.24-16.89 µg/mL. Whereas methoxy and alkyl groups exhibited in the range of low activity around 103.07-872.54 µg/mL. For 3,4-methylenedioxy, it was inactive. In the case of a methyl group, it was noted that the toxicity increased when hydrogen atoms were replaced with fluoro atoms because of the electron withdrawing effect.

In addition, the variation of the positions of the substituents greatly affected the activity. Among bromo substituents, they had tendency of activity from high to low when they were substituted at *para*>*meta*>*ortho*. On the other hand, the activity of methoxy

substituents could be arranged as *ortho> meta> para*, especially *p*-OCH_3 made the activity vividly worse comparing with a reference compound. Moreover, **D29** (R= C_6H_4 (fuse)) and **D1** (R=H) revealed high toxicity with LC_{50}=8.04 and 0.23 μg/mL, respectively.

3.4.2.3 Antibacterial Activity

Seven bacteria which have been known to the general public to cause food poisoning were selected for treating dicoumarols and commercially avaiable antibiotic drugs. These bacteria can be distinguished into three groups: 1) gram-positive bacteria: *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococus aureus*; 2) gram-negative bacteria: *Escherichia coli*, *Escherichia coli* O157:H7 and *Salmonella derby*; and 3) Flat sour spoilage. The method carried out by using paper disc method. Diameter of clear zone was measured after 24 hours of incubation. High inhibition was established when a diameter of clear zone was more than 10 mm. In a case of 7-10 mm diameter of clear zone, weak inhibition was erected. While a diameter of inhibition zone was less than 7 mm, the test compounds were classified as inactive.

Sixteen dicoumarols provided selectively inhibition against four bacteria including *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococus aureus* and Flat sour spoilage. The results are presented as shown in Table 3.16.

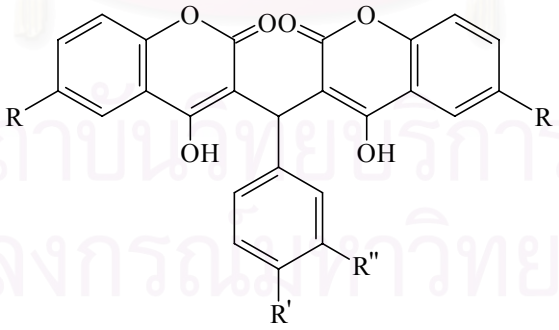


Table 3.16 Antibacterial results of dicoumarols

Group	Cpd	R	R′	R″	B	L	S	FS
I	**D2**	H	H	H	12	15	×	11
	D17	Cl	H	H	15	0	15	15
	D21	CH_3	H	H	8	0	10	8
	D25	OCH_3	H	H	18	0	18	20
II	**D3**	H	NO_2	H	0	0	×	×
	D18	Cl	NO_2	H	10	0	12	12
	D22	CH_3	NO_2	H	15	0	15	15
	D26	OCH_3	NO_2	H	0	0	8	0
III	**D15**	H	OCH_3	H	0	0	0	0
	D19	Cl	OCH_3	H	0	0	8	0
	D23	CH_3	OCH_3	H	0	0	0	0
	D27	OCH_3	OCH_3	H	10	0	8	10
IV	**D16**	H	-OCH_2O-		7	0	×	0
	D20	Cl	-OCH_2O-		0	0	8	0
	D24	CH_3	-OCH_2O-		0	0	0	0
	D28	OCH_3	-OCH_2O-		12	0	8	11
$CHCl_3$					0	0	0	0
MeOH					0	0	0	0
CH_2Cl_2					0	0	0	0
DMSO					0	0	0	0
Streptomycin 10 μg/mL					15	0	17	14
Kanamycin 10 μg/mL					15	20	15	22

Note: B = *Bacillus cereus* L = *Listeria monocytogenes*

S = *Staphylococus aureus* F = Flat sour spoilage

0 = Inactive × = Not tested

From the obtained data, it can be seperated information to four groups by using **D2**, **D3**, **D15** and **D16** as reference compounds of each group, respectively. In the first group, it was observed that dicoumarols with methoxy or chloro substituents at position 6 on a benzopyrone ring displayed high inhibition, a little more than the reference compound. Whereas a dicoumarol with methyl group showed the lowest inhibition activity.

In the second group, all dicoumarols contained nitro group at *para* position on a benzylidene ring but different substituents at position 6 on a benzopyrone ring. Considering of activity against *Bacillus cereus*, a methyl group at position 6 on a benzopyrone ring exhibited the highest activity, more than a chloro group. While a dicoumarol with a methoxy group or without any substituent at the same position was inactive.

In a case of the third group, most of dicoumarols had no activities excepted for **D27** which contained a methoxy group showed weak inhibition. In the last group, it was found that a dicoumarol with a methoxy group revealed higher activity than **D16** which was without substituent at position 6 on benzopyrone ring. Dicoumarols with chloro or methyl substituents at the same position were inactive considering of activity against *Bacillus cereus* and Flat sour spoilage.

From the examination of all results, the methoxy group at position 6 on a benzopyrone ring enhanced inhibition against bacteria but diminished activity when it was disubstituent with nitro group (**D26**). Moreover, **D25** is an interested compound due to the fact that it displayed the highest inhibition activity.

3.2.3 Comparative Studies on Biological Activities of Synthesized Compounds

The selected biological activities performing in this search are of an important role in agricultural and pharmaceutical aspects. In the case of 4-hydroxycoumarins, the result of brine shrimp cytotoxic lethality test was not correlated with weed growth inhibition. Most of them revealed medium cytotoxic activity, whereas they had a little weed growth inhibition except for Compound **3** which exhibited good antigrowth.

However, the consideration of substituent effect was found that a methoxy group is the best substituent increasing two activities of 4-hydroxycoumarins.

Focusing on dicoumarols, all results could be discussed into two categories based on information of three bioassays:

1) Considering brine shrimp cytotoxic lethality test and antibacterial activity

Two activities had correlation in substituent effect, particularly the substituents at position 6 on a benzopyrone ring. Referring to the obtained data, it could be indicated that a methoxy group displayed the highest activity among other substituents. Although a methoxy group exhibited low cytotoxic activity, it revealed high antibacterial activity.

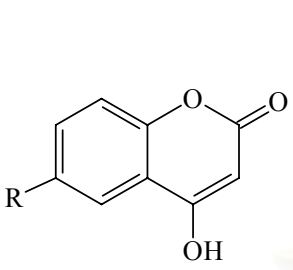
2) Contemplating brine shrimp cytotoxic lethality test and acute toxicity assay

In the study of cytotoxic activity, this research evaluated the activity from arthropod to vertebrates having complicated organism. It was noted that the result of brine shrimp cytotoxic lethality test was not correlated with the acute toxicity assay because there is the difference in the metabolite of each animal. The dicoumarols with substituents on a benzylidene ring showed toxicity against brine shrimp in medium level, while they exhibited toxicity against Nile tilapia from low to high level. Regarding to types of substituent, the nitro group is the best one providing high toxicity against Nile tilapia with LC_{50} = 0.24 μg/mL which closed to that of standard (1,4-naphthoquinone, LC_{50} = 0.11 μg/mL).

On the other hand, the 3,4-methylenedioxy substituent showed low toxicity against brine shrimp and had no activity against Nile tilapia. Whereas it expressed interesting antifeedant activity against *Galleria mellonella* (55%). In the similar results, the methoxy and isopropyl substituents displayed low toxicity against Nile tilapia, but they revealed quite high and moderate antifeedant activity (82% and 51%, respectively).[17]

From the results of brine shrimp lethality test, the relationship between substituent and activity was compared among synthesized compounds including 4-hydroxycoumarins and dicoumarols. Regarding substituents at position 6 on a benzopyrone ring, 4-hydroxycoumarins exhibited higher activity than dicoumarols. Even though dicoumarols comprised other substituents on a benzylidene ring, the activity remained less than 4-hydroxycoumarins, except for compound **D19**. The obtained

information implied that the activity was depended on size of structure. To illustrate this, the monomer molecule displayed better activity than the dimer molecule. The comparative data is presented in Table 3.17.



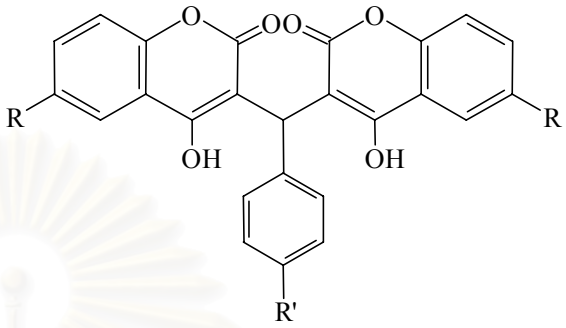
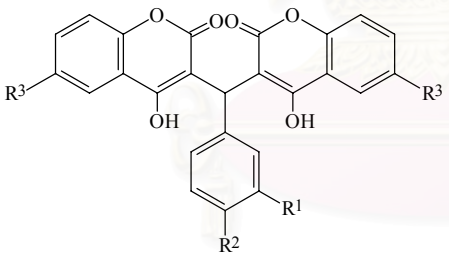


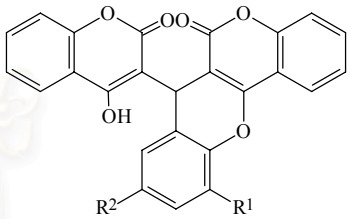
Table 3.17 The comparative results of the LC_{50} values of 4-hydroxycoumarins and dicoumarols

R	4-Hydroxycoumarin		Dicoumarol		
	Cpd	LC_{50} (μg/mL)	R′	Cpd	LC_{50} (μg/mL)
CH_3	**2**	37.26	H	**D21**	761.66
			NO_2	**D22**	1028.59
			OCH_3	**D23**	368.66
			$-OCH_2O-$	**D24**	922.85
OCH_3	**4**	19.21	H	**D25**	50.54
			NO_2	**D26**	91.60
			OCH_3	**D27**	47.15
			$-OCH_2O-$	**D28**	99.84
Cl	**6**	43.73	H	**D17**	385.67
			NO_2	**D18**	260.02
			OCH_3	**D19**	31.03
			$-OCH_2O-$	**D20**	784.13

CHAPTER IV
CONCLUSION

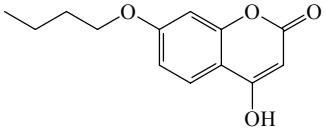
This research purposed to comprehend the effect of substituents of 4-hydroxycoumarins, dicoumarols and related compounds on their activities. 4-Hydroxycoumarins could be achieved by utilizing the condensation of 2-hydroxyacetophenone and diethyl carbonate with sodium hydride. Condensation of 2 mol equivalents of 4-hydroxycoumarin with various aldehydes provided substituted dicoumarols and fused-ring compounds. All synthesized compounds were well characterized using their physical properties and spectroscopic techniques such as IR, ^{1}H- and ^{13}C-NMR, and in some cases MS were also carried out. There are sixteen compounds including **12**, **D18**, **D20**, **D22**, **D24**, **D25**, **D26**, **D27**, **D28**, **D32**, **D34**, **D35**, **D36**, **D37**, **D38** and **D39** that have not been reported in chemical literatures. The structures of new compounds are shown as the following:



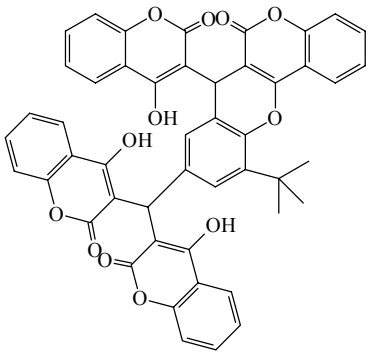


Cpd	R^1	R^2	R^3
D18	H	NO_2	Cl
D20	-OCH_2O-		Cl
D22	H	NO_2	CH_3
D24	-OCH_2O-		CH_3
D25	H	H	OCH_3
D26	H	NO_2	OCH_3
D27	H	OCH_3	OCH_3
D28	-OCH_2O-		OCH_3

Cpd	R^1	R^2
D32	CHO	OCH_3
D34	CHO	CH_3
D35	$C(CH_3)_3$	CH_3
D36	H	$C(CH_3)_3$
D37	$C(CH_3)_3$	$C(CH_3)_3$
D38	$C(CH_3)_3$	OCH_2CH_3



12



D39

In this study, the synthesized compounds were screened among four bioassays: brine shrimp cytotoxic lethality test, weed growth inhibition test, static acute toxicity bioassay and antibacterial activity. A methoxy group is the most interesting substituent in brine shrimp lethality test. Both of 4-hydroxycoumarins and dicoumarols bearing the methoxy group almost exhibited better activity than other substituents. Considering the substituents at position 6 on benzopyrone ring of 4-hydroxycoumarins and dicoumarols, it could be summarized that monomer molecules displayed higher activity than dimer molecules. This implied that 4-hydroxycoumarins are more attractive for further study than dicoumarols. In case of fused-ring compounds, the tertiary butyl substituent enhanced the activity, while the carboxaldehyde substituent decreased it.

Focusing on weed growing inhibition against *M. pigra*, the obtained results could confirm the previous conclusion[17] that almost 4-hydroxycoumarins did not show any distinguished activity, except for **3**. This compound displayed activity as good as or a little lower than commercially available herbicides. Comparing the methoxy substituent at various positions, 5-OCH_3 showed the best activity, where 6- and 7-OCH_3 were not active.

Contemplating in the case of acute toxicity against *O. niloticus*, dicoumarols with electron withdrawing groups exhibited higher toxicity than dicoumarols having electron donating groups. The nitro group is the best one comparing with other substituents at *p*-position on benzylidene. Although the 3,4-methylenedioxy group has no acute toxicity, it is an interesting compound for further study as a non-toxic substance.

Antibacterial activity is the other bioassay to which substituted dicoumarols were also subjected. These compounds were found to be selectively active with four food-poisoning bacteria, namely, *Bacillus cereus*, *Listeria monocytogenes*,

Staphylococus aureus and Flat sour spoilage. Dicoumarols with a methoxy group at position 6 on a benzopyrone ring (**D25**) displayed the highest inhibition.

This study revealed that the substituents affected directly to the activity and the synthesized compounds showed broad spectrum activity. For acute toxicity against *O. niloticus* of these compounds, it has never been reported in literatures before. This preliminary screening utilized for the development of more effective pharmaceuticals and agrochemicals in the future.

Proposal for the future work

The biological activities of 4-hydroxycoumarins and dicoumarols should be investigated continuously. In the case of cytotoxic activity, brine shrimp lethality test was the based information which could be used for further study on other bioassays. Acute toxicity test should be evaluated to another animals. The antibacterial activity should be proceeded to obtain complete results. The study of substituent effect of these compounds was still attractive to be searched for more active compounds.

REFERENCES

1. Murray, R. D. H.; Mendez, J.; Brown, S. A. *The Natural Coumarins*, John Wiley & Sons, Inc., New York, **1982**, 1.
2. Chen, Y. L.; Wang, T. C.; Lee, K. H.; Tzeng, C. C.; Chang, Y. L.; Teng, C. M. "Synthesis of Coumarin Derivatives as Inhibitors of Platelet Aggregation", *Helv. Chim. Acta*, **1996**, *79*, 651.
3. Lammaouer, D.; Fraigui, O.; Martin, M. T.; Bodo, B. "Structure of Ferulenol Derivatives from *Ferula communis* var. *genuina*", *Phytochemistry*, **1991**, *30*, 2383.
4. Stahmann, M. A.; Wolff, I.; Link, K. P. "Studies on 4-Hydroxycoumarins. I. The Synthesis of 4-Hydroxycoumarins", *J. Am. Chem. Soc.*, **1943**, *65*, 2285.
5. Shah, V. R.; Base, J. L.; Shah, R. C. "New Synthesis of 4-Hydroxycoumarins", *J. Org. Chem.*, **1960**, *25*, 677.
6. Thaisrivongs, S.; Watenpaugh, K. D. *et al*. "Structure-Based Design of Novel HIV Protease Inhibitors: Carboxamide-Containing 4-Hydroxycoumarins and 4-Hydroxy-2-pyrones as Potent Nonpeptidic Inhibitors", *J. Med. Chem.*, **1995**, *38*, 3624.
7. Buckle, D. R.; Cantello, B. C. C.; Smith, H.; Spicer, B. A. " Antiallergic Activity of 4-Hydroxy-3-nitrocoumarins", *J. Med. Chem.*, **1975**, *18*, 391.
8. Heerden, P. S.; Benzuidenhoudt, B. C. B.; Ferreira, D. "Improved synthesis for the rodenticides, diphenacoum and brodifacoum", *J. Chem. Soc., Perkin, Trans. 1*, **1997**, 1141.
9. Jung, J. C.; Kim, J. C.; Park, O. S. "Simple and cost effective syntheses of 4-hydroxycoumarin", *Synth. Comm.*, **1999**, *29*, 3587.
10. Sullivan, W. R.; Huebner, C. F.; Stahmann, M. A.; Link, K. P. "Studies on 4-Hydroxycoumarins. II. The Condensation of Aldehydes with 4-Hydroxycoumarins", *J. Am. Chem. Soc.*, **1943**, *65*, 2288.
11. Litvan, F.; Stoll, W.G. "Uber Ringschlussreaktionen von 3,3′-Methylen-bis-(4-hydroxycoumarinen)", *Helv. Chim. Acta*, **1959**, *42*, 878.

12. Hayward, R. C. "The Prototype Compound for the Oral Anticoagulants 3,3′-methylene bis(4-hydroxycoumarin), *J. Chem Edu.*,**1984**, *61*, 87.

13. Appendino, G.; Cravotto, G.; Tagliapietra, S.; Nano, G. M.; Palmisano, G. "The Chemistry of Coumarin Derivatives", *Helv. Chim. Acta*, **1990**, *73*, 1865.

14. Dall,-Larsen, T.; Kryvi, H.; Klungsoyr, L. "Dinitrophenol, Dicoumarol and Pentachlorophenol as Inhibitors and Parasite Substrates in the ATP Phosphoribosyltransferase Reaction", *J. Biochem.*, **1976**, *66*, 443.

15. Wang, S.; Milne, G. W. A.; Yan, X.; Posey, I. J.; Nicklaus, M. C.; Graham, L.; Rice, W. G. "Discovery of Novel, Non-Peptide HIV-1 Protease Inhibitors by Pharmacophore Searching", *J. Med. Chem.*, **1996**, *39*, 2047.

16. Zhao, H.; Neamatis, N.; Pommiers, Y.; Burke, T. R. " Design and Synthsis of Photoactivatable Coumarin-Containing HIV-1 Integrase Inhibitors", *Heterocycles*, **1997**, *45*, 2277.

17. Wattanasereekul, S. *Structure-Activity Relationship of 4-Hydroxycoumarins and Related Compounds*, Master's Thesis, Department of chemistry, Graduate School, Chulalongkorn University, 1998.

18. Sirisuksukon, W. *Synthesis of 3-Substituted-4-hydroxycoumarins and their Biological Activities*, Master's Thesis, Department of chemistry, Graduate School, Chulalongkorn University, 1999.

19. Tietze, L.; Eicher, T. *Reaction and Synthesis in the Organic Chemistry Laboratory*, University Science Books Mill Valley, California, **1989**, 122.

20. Dictionary of Organic Compounds, 4th ed., Completely revised, enlarge and re-set edition in five volumes: Eyre & Spolttis woode, Publishers Ltd. E & F. N. SPON Ltd.

21. Martin, R. "Uses of the Fries Rearrangement for the Preparation of Hydroxyarylketones. A Review", *Organic Prepararions and Procedures Int.,***1992**, *24*, 369.

22. Hermodson, M. A.; Barker, W. M.; Link, K. P. "Studies on the 4-Hydroxycoumarins. Synthesis of the Metabolites and Some Other Derivatives of Warfarin", *J. Med. Chem.*, **1971**, *14*, 167.

23. Heller, H.; Oliver, S.; Whittall, J.; Tomlinson, I. "Photochromic spiropyran compounds" : U.S. Pat. 4,826,977 May.02.1989.

24. Beger, J.; Binte, H. J.; Brunne, L.; Neumann, R. "Synthesis of 4-alkoxy-2-hydroxyphenyl ketoximes as reagents for metal extraction", *J. Prakt. Chem./Chem.-Ztg.*, **1992**, *334*, 269.

25. a) Bhanu, S.; Saroja, T.; Seshadri, T. R.; Mukerjee, S. K. *Indian J. Chem.*, **1972**, *10*, 16. b) Desai, N. J.; Sethna, S. *Synthesis*, **1957**, *22*, 388. c) Bhanu, S.; Saroja, T.; Seshadri, T. R.; Mukerjee, S. K. *Indian, J. Chem.*, **1971**, *9*, 380.

26. Domagala, J. M.; Hagen, S. E.; Lunney, E.; Tait, B. D. "Coumarin derivatives as HIV protease inhibitors and antiviral agents" : U.S. Pat 5,510,375 Apr.23.1996.

27. Larrow, J. F.; Jacobsen, E. N.; Gao, Y.; Hong, Y.; Nie, X.; Zepp, C. M. "A Practical Method for the Large-Scale Preparation of [*N*,*N*′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato (2-)]manganese (III) Chloride, a Highly Enantioselective Epoxidation Catalyst", *J. Org. Chem.*, **1994**, *59*,1939.

28. Klosa, *J. Apoth-Ztg.*, **1952**, *4*, 55.

29. Inescu, C. N.; Selmiciu, I.; Niculescu, V.; Gostea, T.; Leoveanu, O. "Synthetic Anticoagulants", *Acad. Rep. Populare Romine, Studii Cercetari Chim.*, **1954**, *2*, 195.

30. Arora, R. B.; Krishnaswamy, N. R.; Seshadri, T. R.; Setch, S. D. S.; Sharma, B. R. *Indian J. Chem.*, **1967**, *10*, 121.

31. Klosa, J. "Synthesis of 4-Hydroxy-6-chlorocoumarins derivatives", *Arch. Pharm.*, **1956**, *289*, 143.

32. Eckstein, M.; Sulko, J. "Derivatives of 4-Hydroxycoumarin with Substituted Salicylaldehydes", *Ann. Chim.*, **1965**, *55*, 365.

33. Solis, P. N.; Wright, C. W.; Anderson, M. M.; Gupta, M. P.; Phillipson, J. D. A. "Microwell Cytotoxicity Assays Using *Artemia salina* (Brine Shrimp)", *Planta Med.*, **1993**, *59*, 250.

34. Harada, J.; Yano, M. *Proceedings of the Ninth Asian-Pacific Weed Science Society Conference*, The nation science and Technology Authority and Philippine Tobacco Research and Training Center, **1983**, 71.

35. American Society for Testing and Materials: Standard Practice for Conducting Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. ASTM 729-80. Philadelphia: ASTM

36. Myszka, D. G.; Swenson, R. P. "Synthesis of the Photoaffinity Probe 3-(*p*-Azidobenzyl)-4-hydroxycoumarin and Identification of the Dicoumarol Binding Site in Rat Liver NAD(P)H: Quinone Reductase (EC 1.6.99.2)", *J. Biol. Chem.*, **1991**, *266*, 4789.

37. Tummino, P. J.; Ferguson, D.; Hupe, L.; Hupe, D. "Competitive inhibition of HIV-1 Protease by 4-Hydroxy benzopyran-2-ones and by 4-hydroxy-6-phenylpyran-2-ones", *Biochem. Biophys. Res. Comm.*, **1994**, *200*, 1658.

38. Romines, K. R.; Watenpaugh, K. D. *et al*. "Use of Medium-Sized Cycloalkyl Rings To Enhance Secondry Binding: Discovery of a New Class of Human Immunodeficiency Virus (HIV) Protease Inhibitors", *J. Med. Chem*., **1995**, *38*, 1884.

39. Mazumder, A.; Wang, S.; Neamati, N.; Nicklaus, M.; Sunder, S.; Chen, J.; Milne, G. W. A.; Rice, W. G.; Burke, T.R.; Pommier, Y. "Antiretroviral Agents as Inhibitors of Both Human Immunodeficiency Virus Type 1 Integrase and Protease", *J. Med. Chem.*, **1996**, *39*, 2472.

40. Parmer, V. S.; Bisht, K. S.; Jain, R.; Singh, S.; Sharma, S. K.; Gupta, S.; Malhotra, S.; Tyagi, O. D.; VardHan, A.; Pati, H. N.; Berghe, D. V.; Vlietnck, A. J. "Synthesis, Antimicrobial and Antiviral Activities of Novel Polyphenolic Compounds", *Indian J. Chem.*, **1996**, *35(B)*, 220.

41. Yang, B.; Sutcliffe, J.; Dutton, C. J. " 4-Hydroxycoumarin Derivatives with Antibacterial Activity" : U.S. Pat. 5,624,951 Apr.29.1997.

APPENDIX

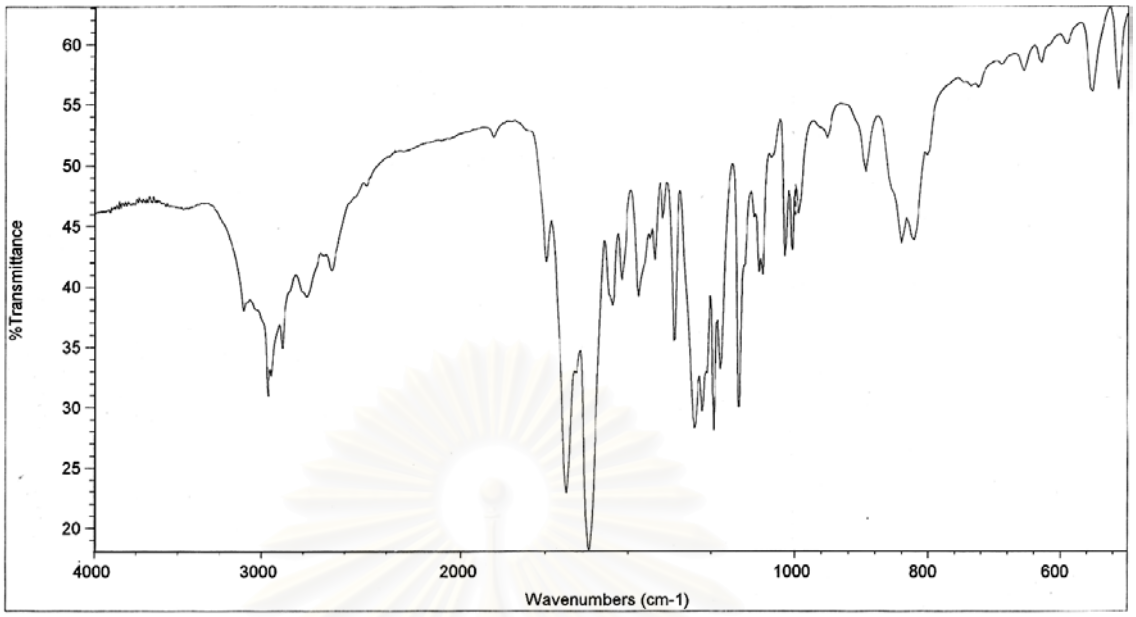


Fig 1 The FT-IR spectrum of Compound **12**

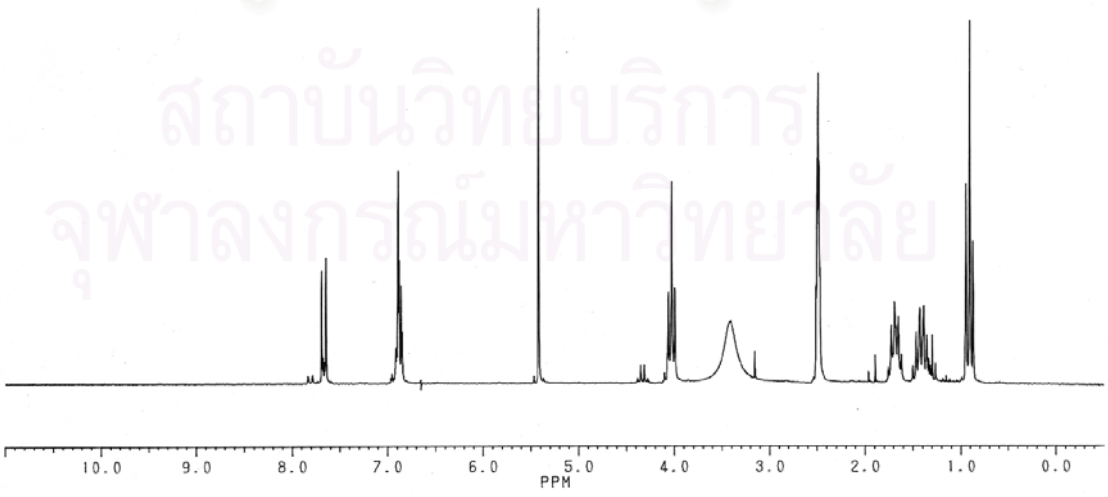


Fig 2 The ^{1}H-NMR spectrum of Compound **12**

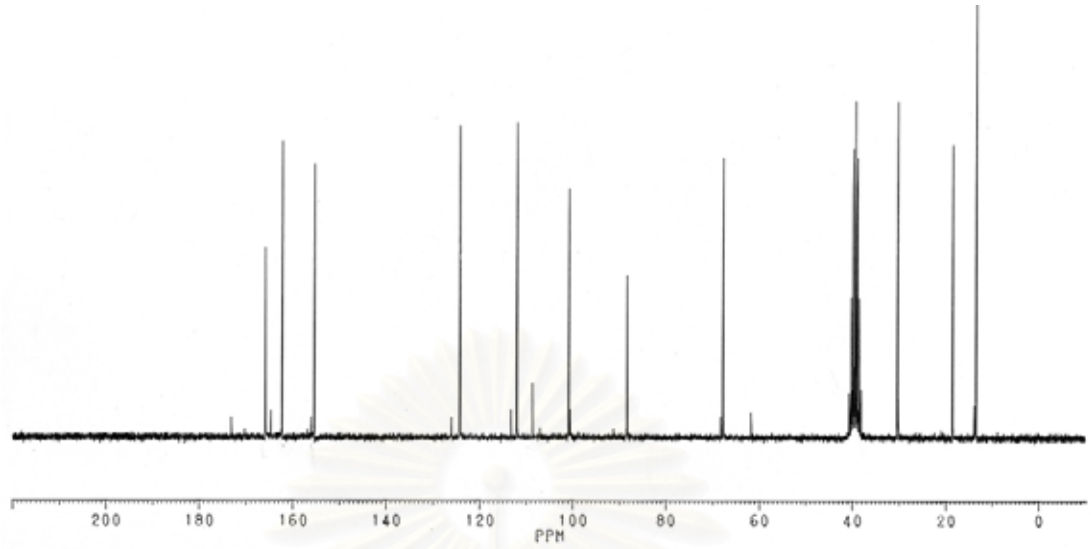


Fig 3 The ^{13}C-NMR spectrum of Compound **12**

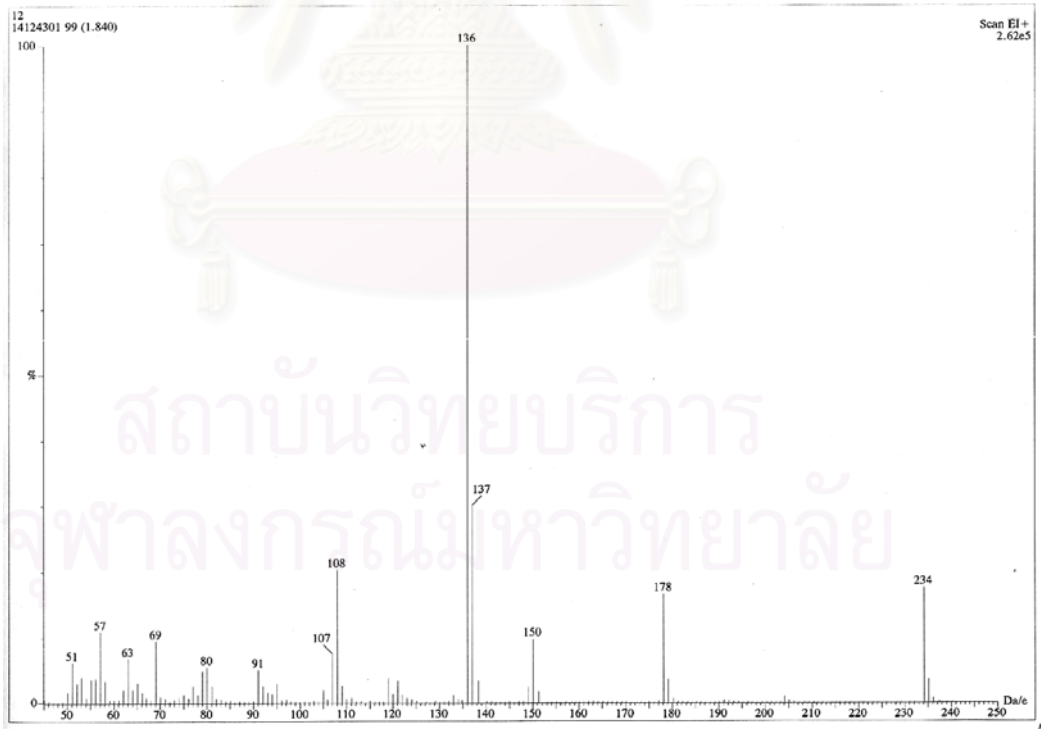


Fig 4 The mass spectrum of Compound **12**

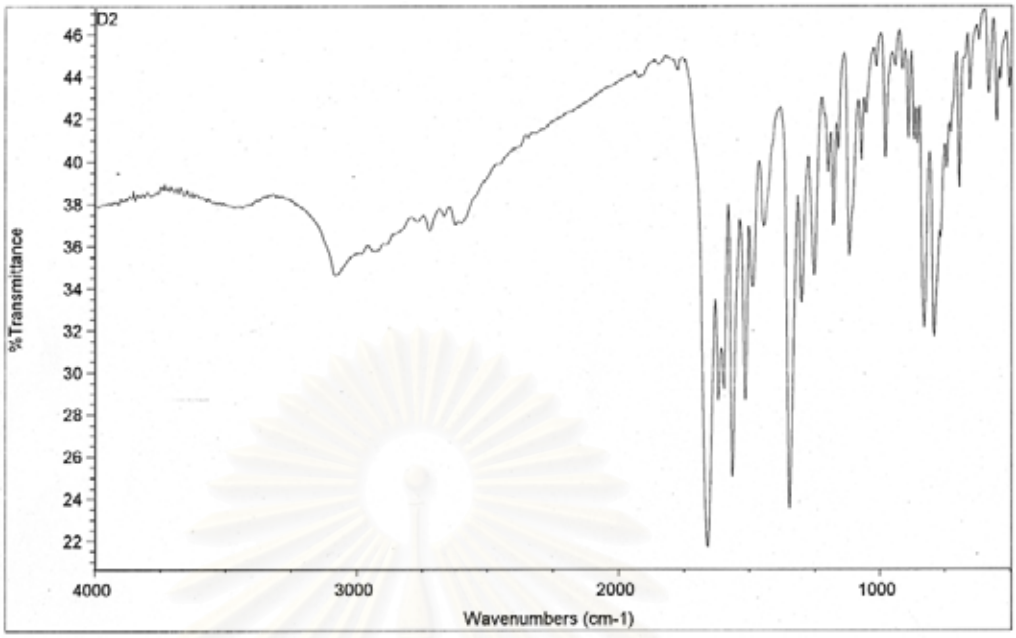


Fig 5 The FT-IR spectrum of Compound **D18**

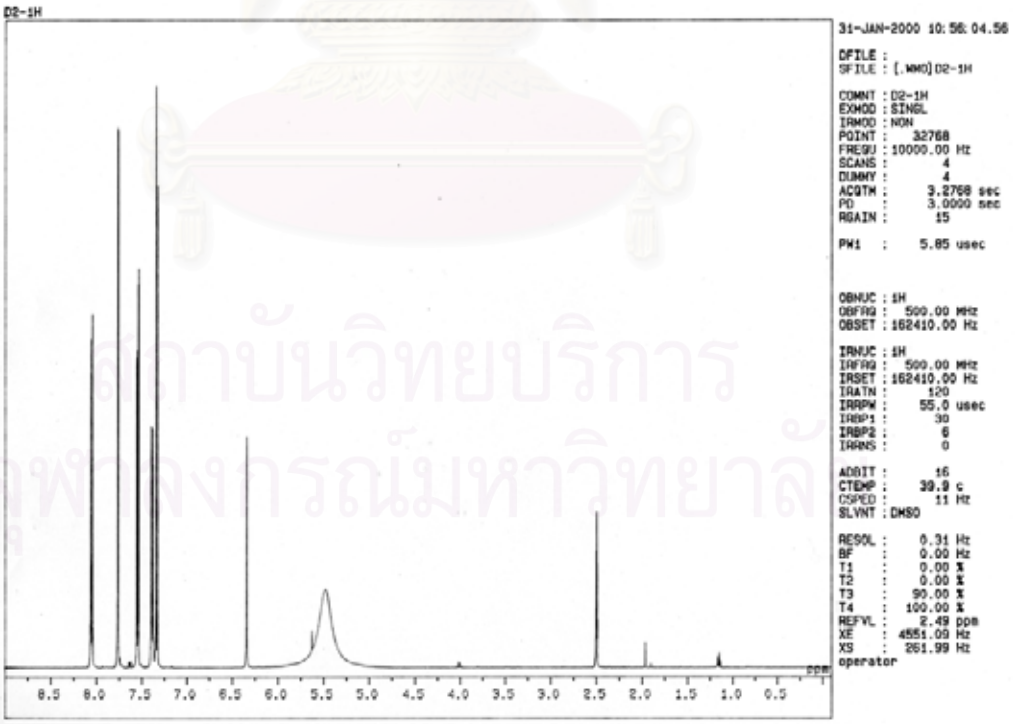


Fig 6 The ^{1}H-NMR spectrum of Compound **D18**

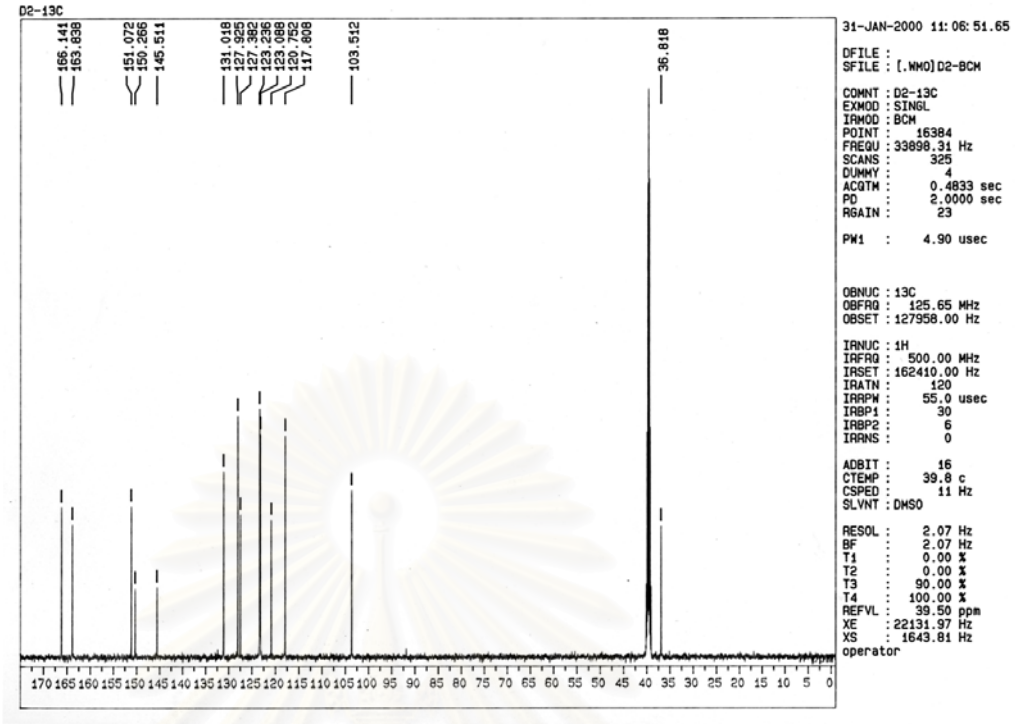


Fig 7 The ^{13}C-NMR spectrum of Compound **D18**

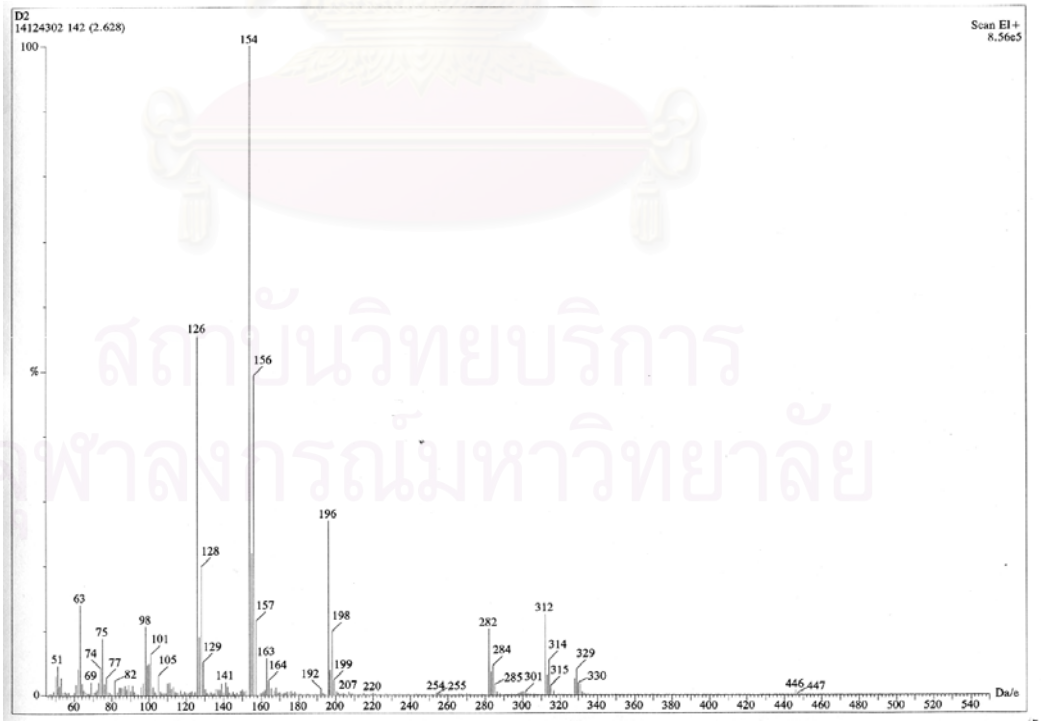


Fig 8 The mass spectrum of Compound **D18**

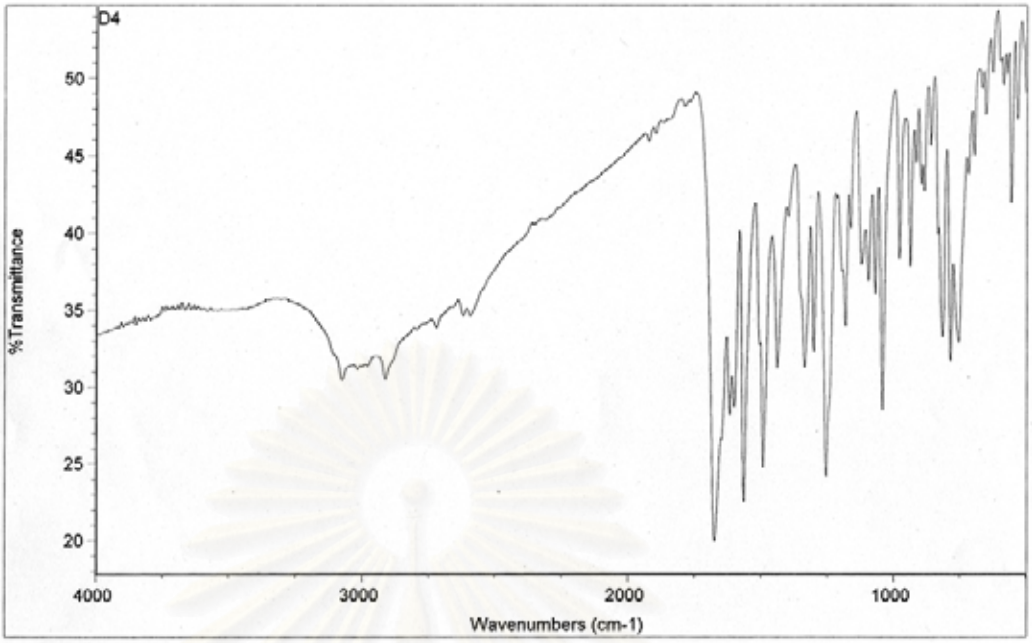


Fig 9 The FT-IR spectrum of Compound **D20**

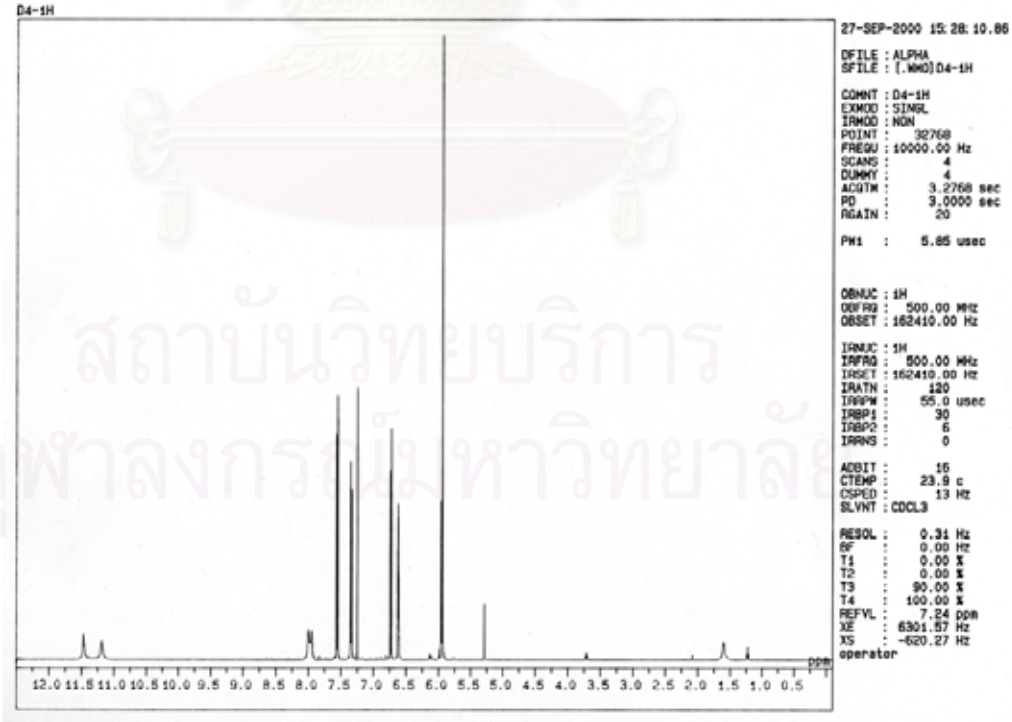


Fig 10 The ^{1}H-NMR spectrum of Compound **D20**

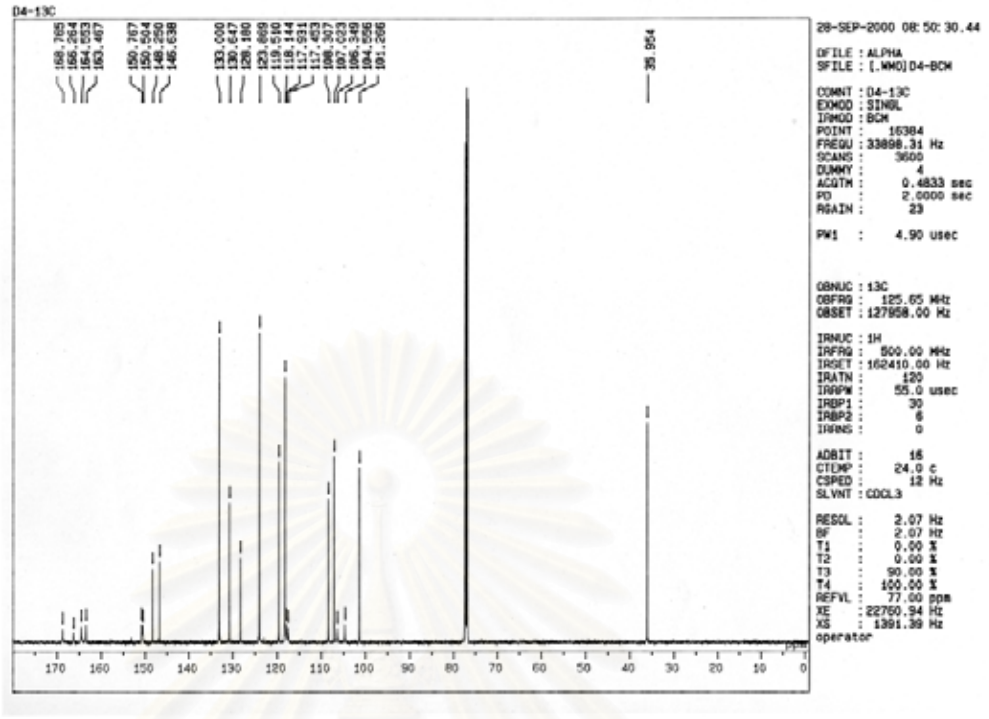


Fig 11 The ^{13}C-NMR spectrum of Compound **D20**

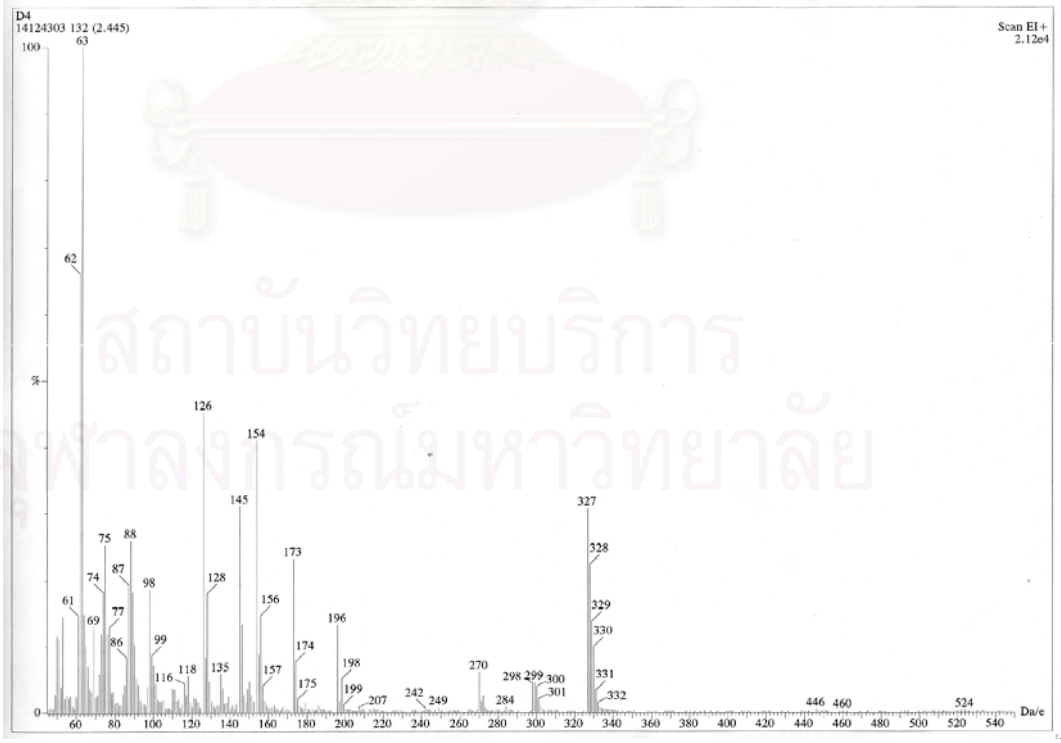


Fig 12 The mass spectrum of Compound **D20**

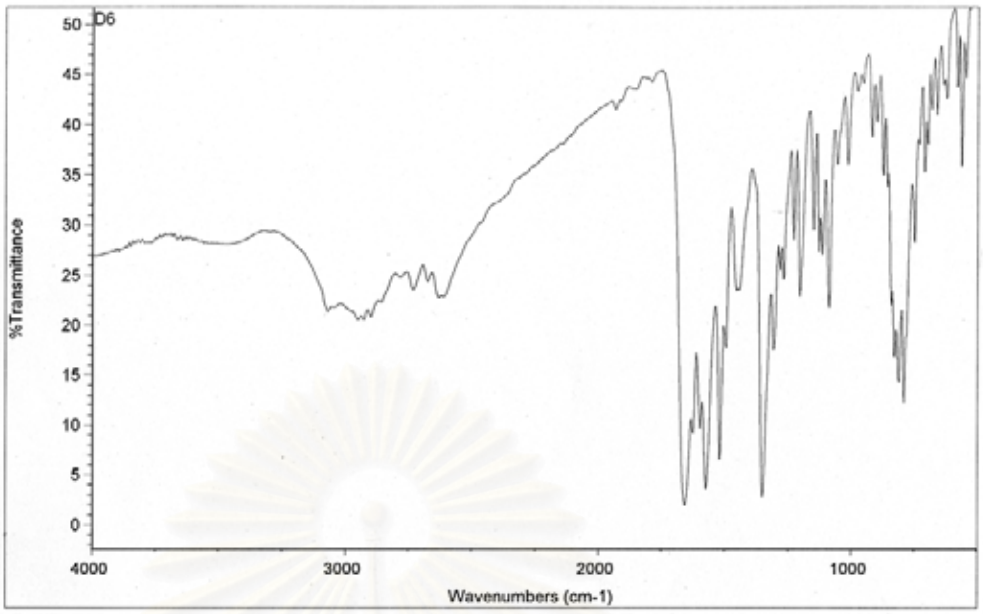


Fig 13 The FT-IR spectrum of Compound **D22**

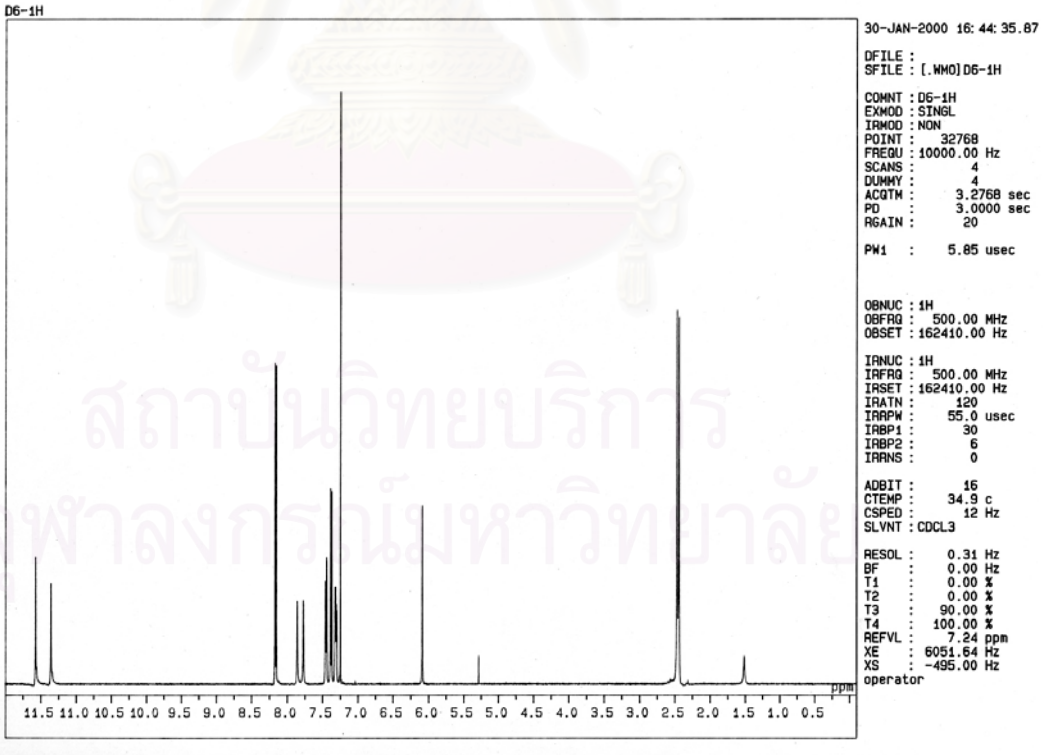


Fig 14 The ^{1}H-NMR spectrum of Compound **D22**

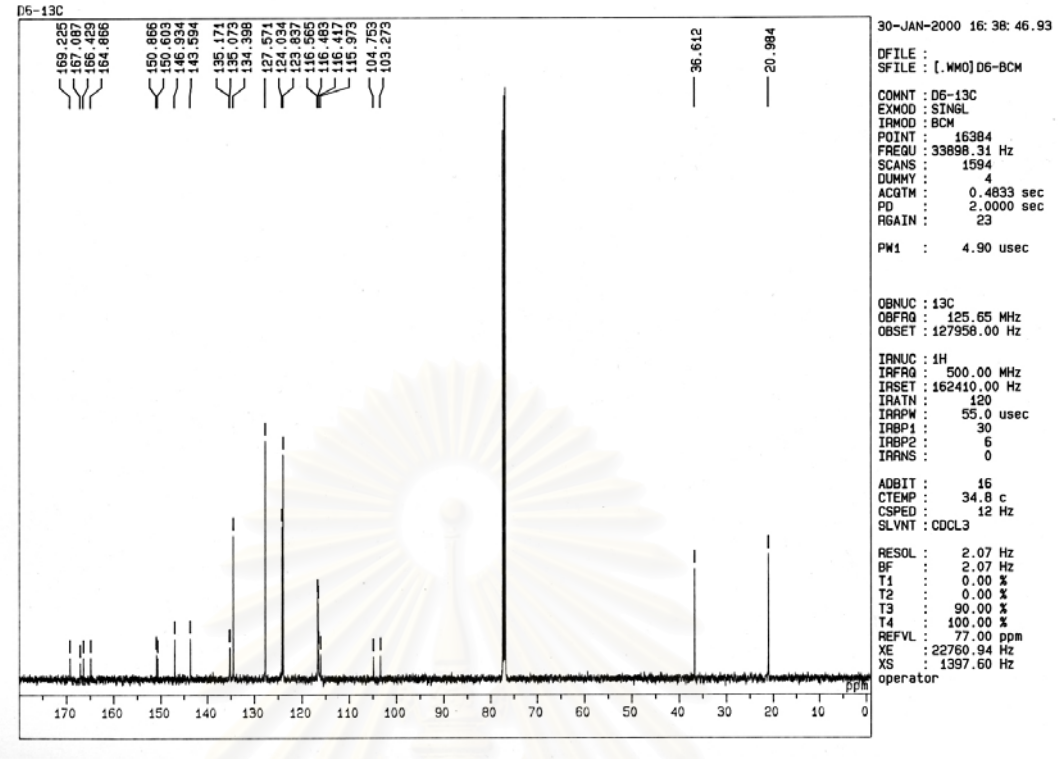


Fig 15 The of ^{13}C-NMR Compound **D22**

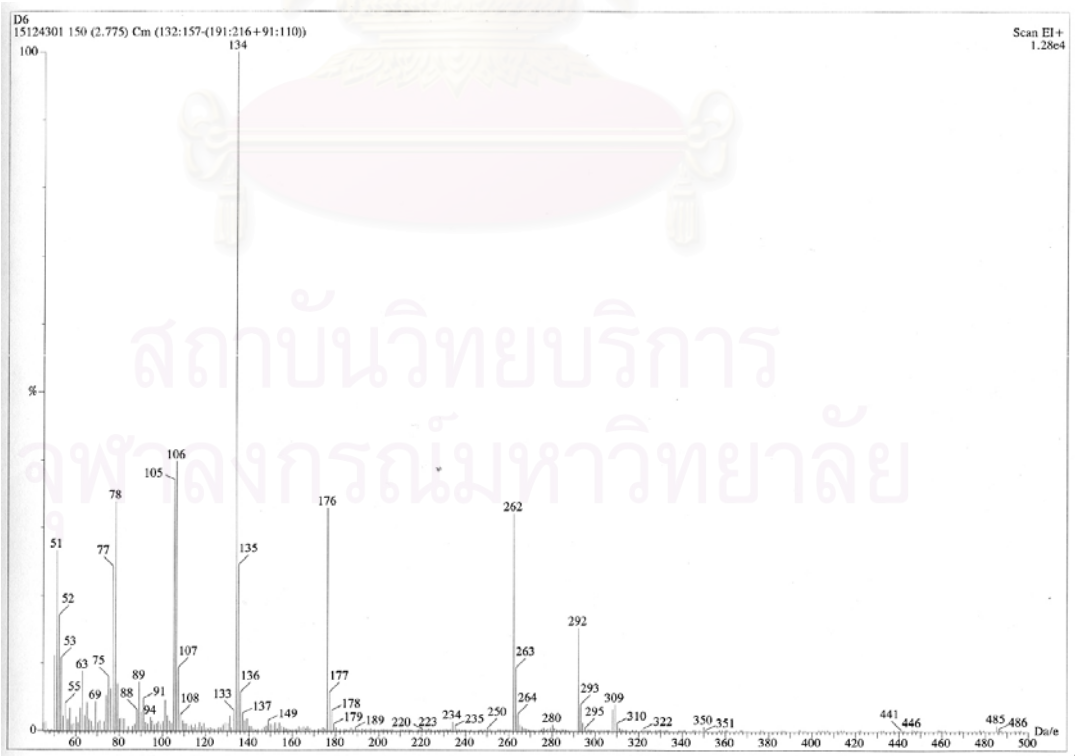


Fig 16 The mass spectrum of Compound **D22**

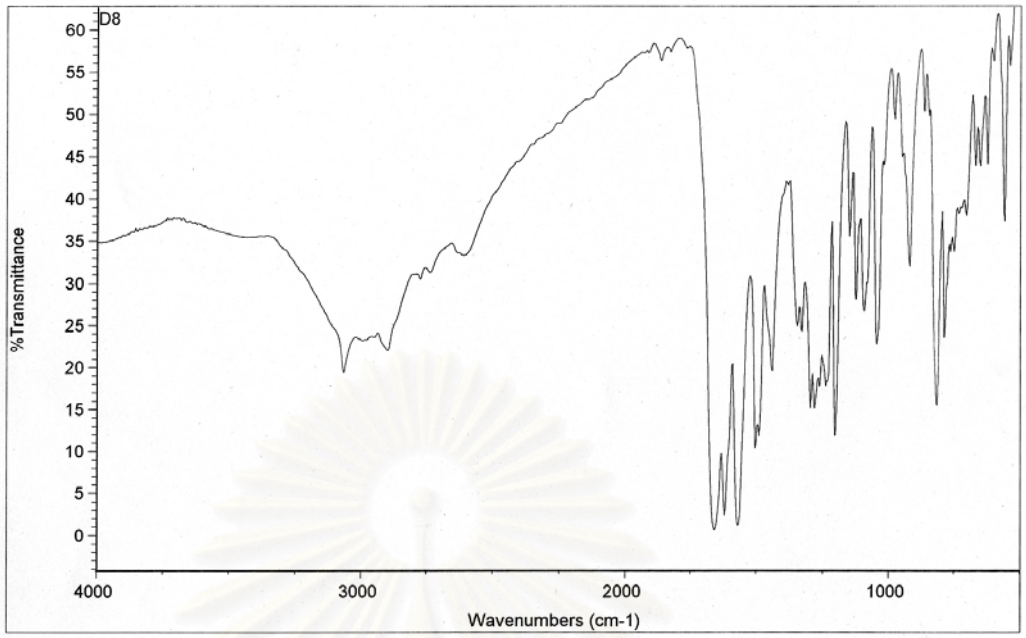


Fig 17 The FT-IR spectrum of Compound **D24**

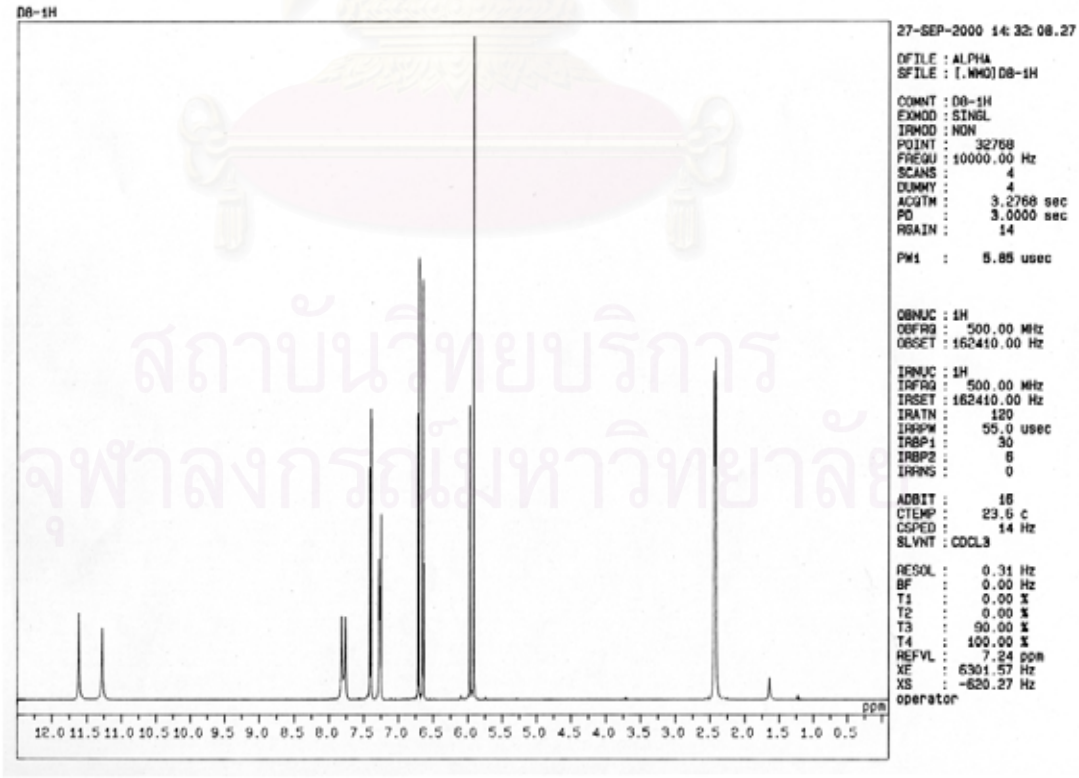


Fig 18 The ^{1}H-NMR spectrum of Compound **D24**

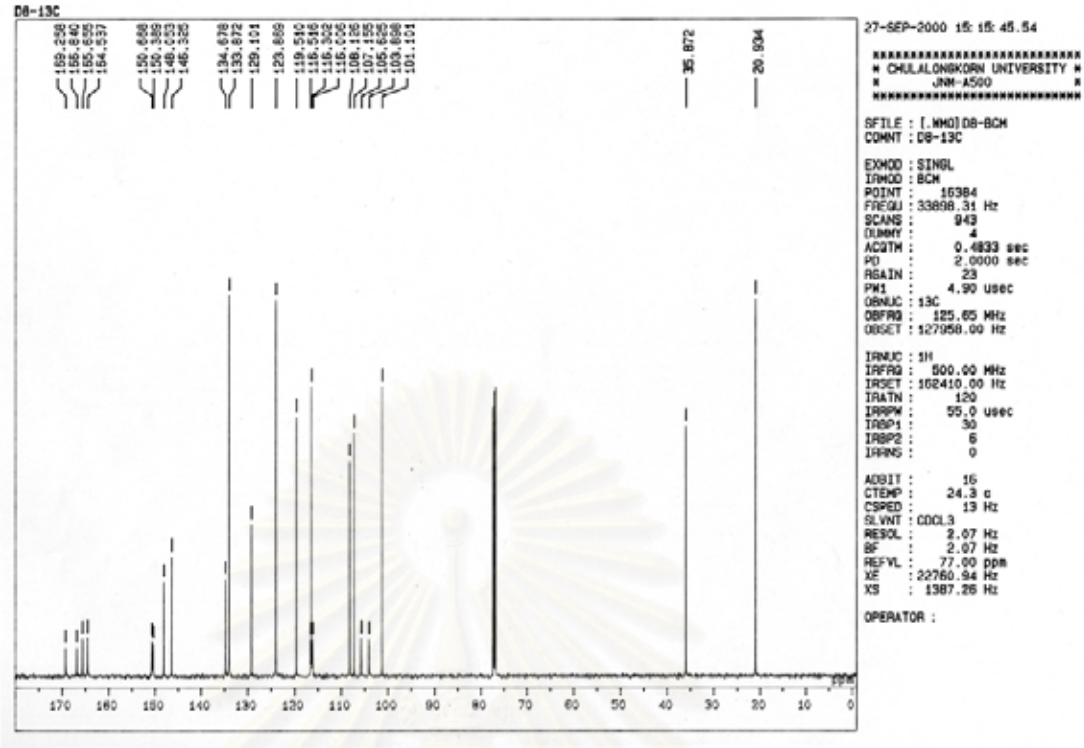


Fig 19 The of ^{13}C-NMR Compound **D24**

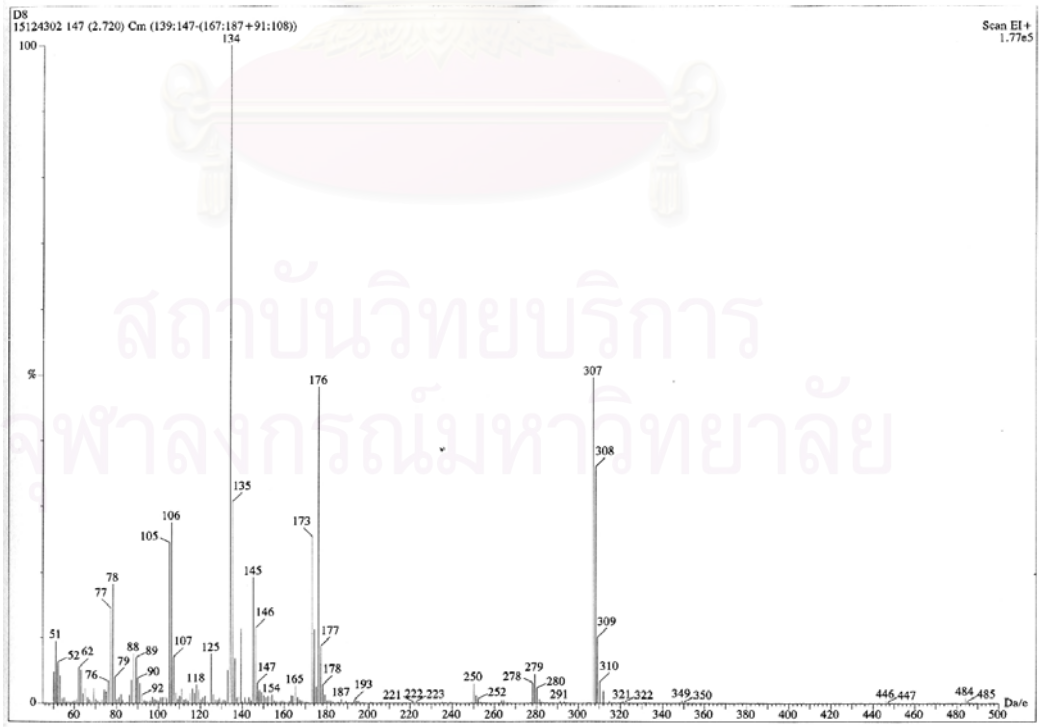


Fig 20 The mass spectrum of Compound **D24**

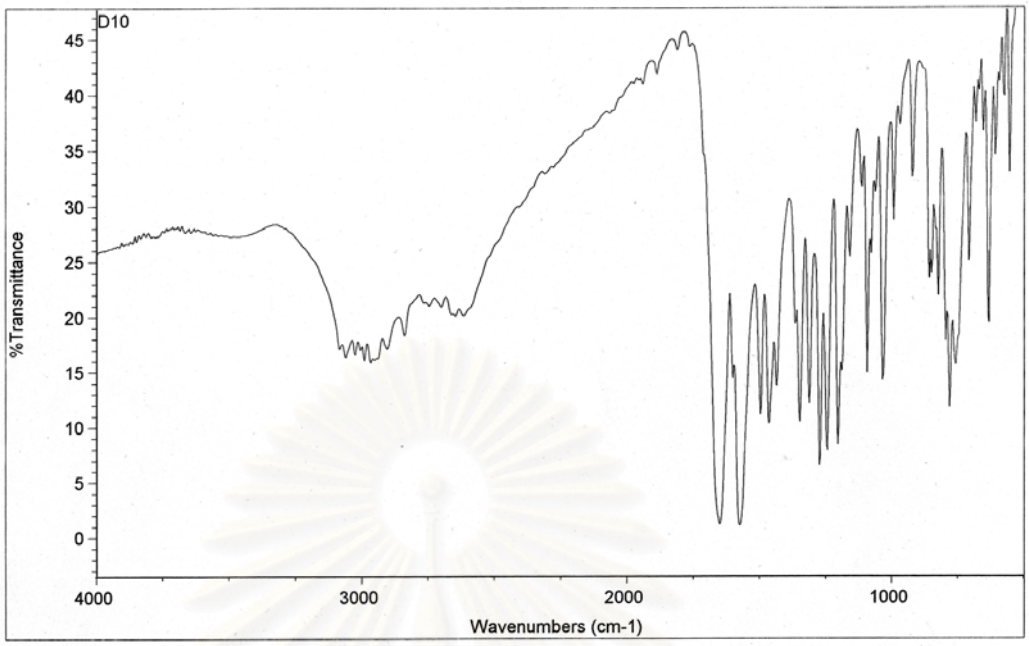


Fig 21 The FT-IR spectrum of Compound **D25**

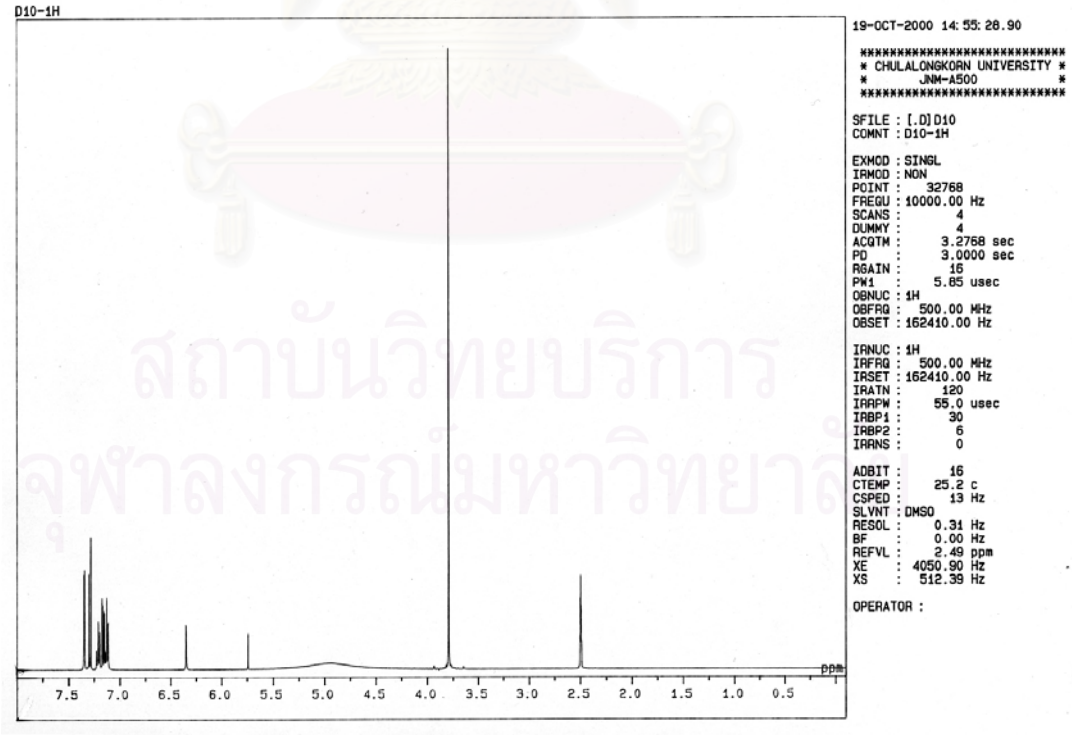


Fig 22 The ^{1}H-NMR spectrum of Compound **D25**

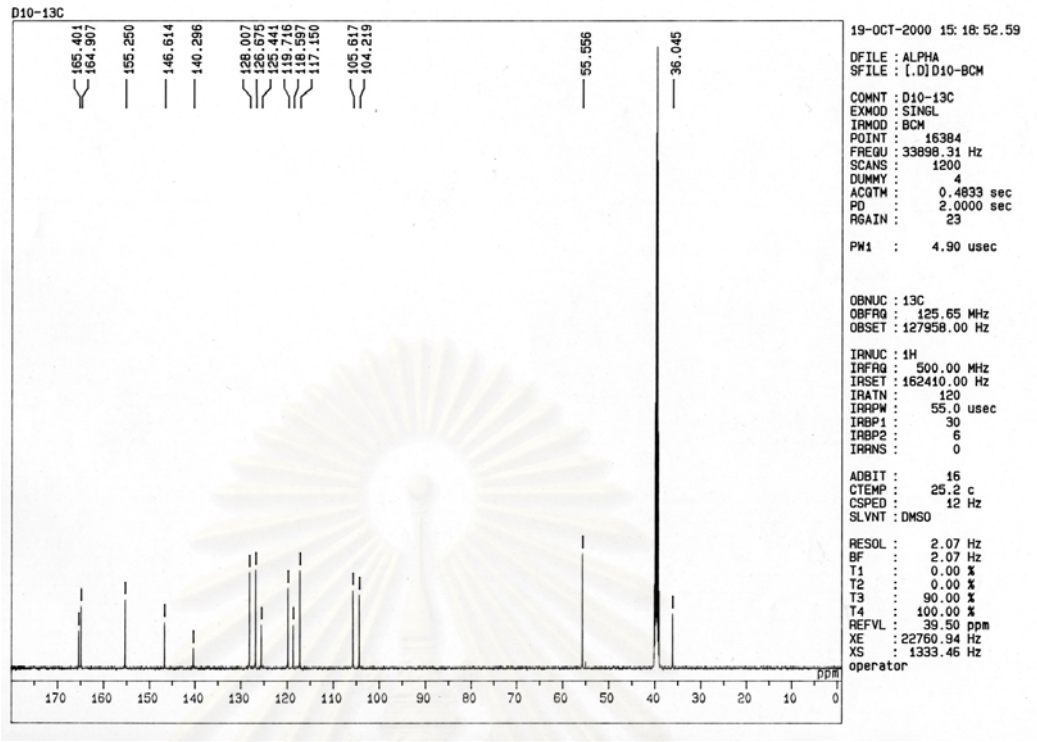


Fig 23 The ^{13}C-NMR spectrum of Compound **D25**

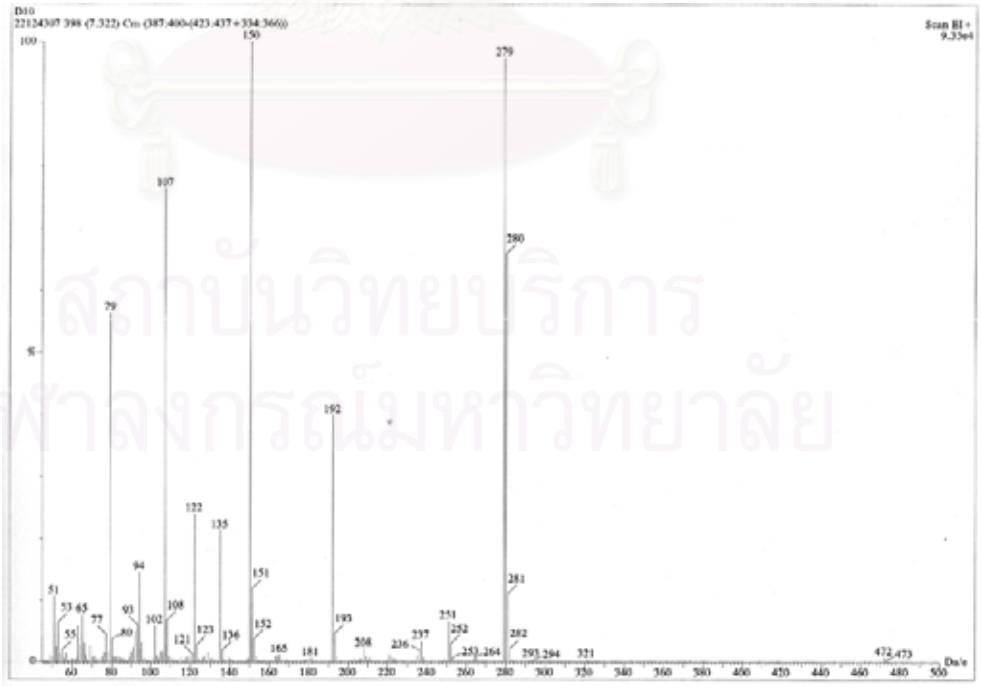


Fig 24 The mass spectrum of Compound **D25**

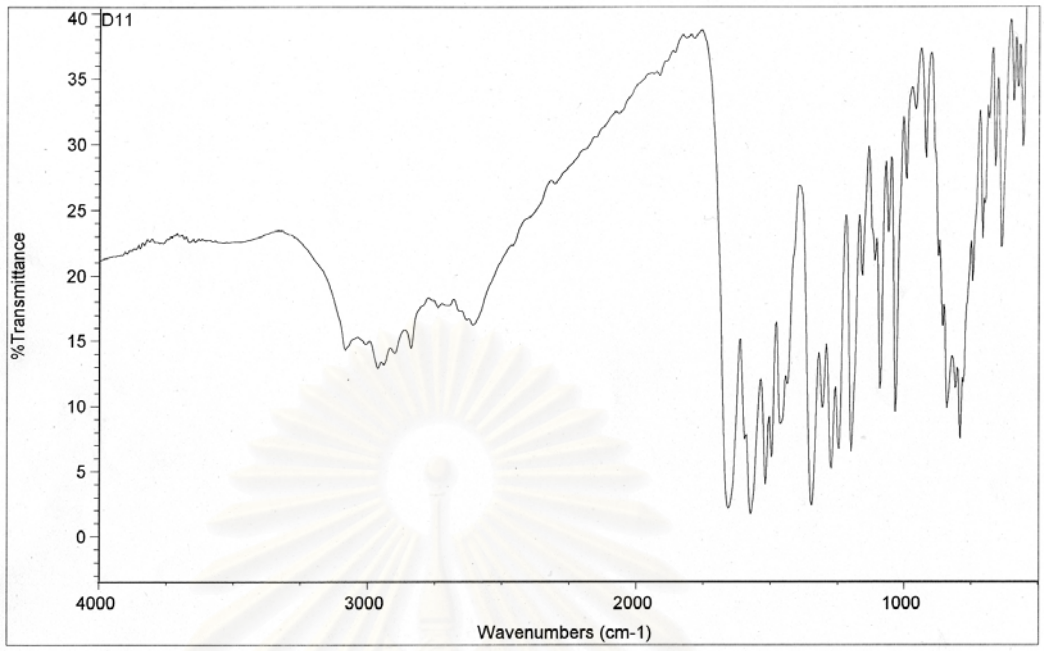


Fig 25 The FT-IR spectrum of Compound **D26**

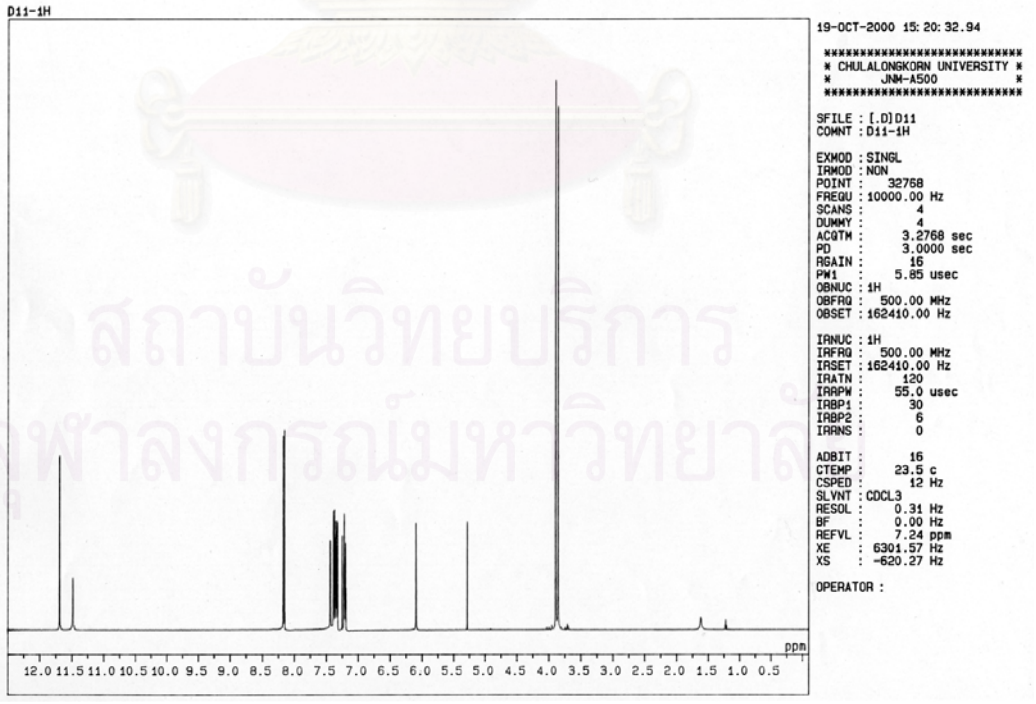


Fig 26 The ^{1}H-NMR spectrum of Compound **D26**

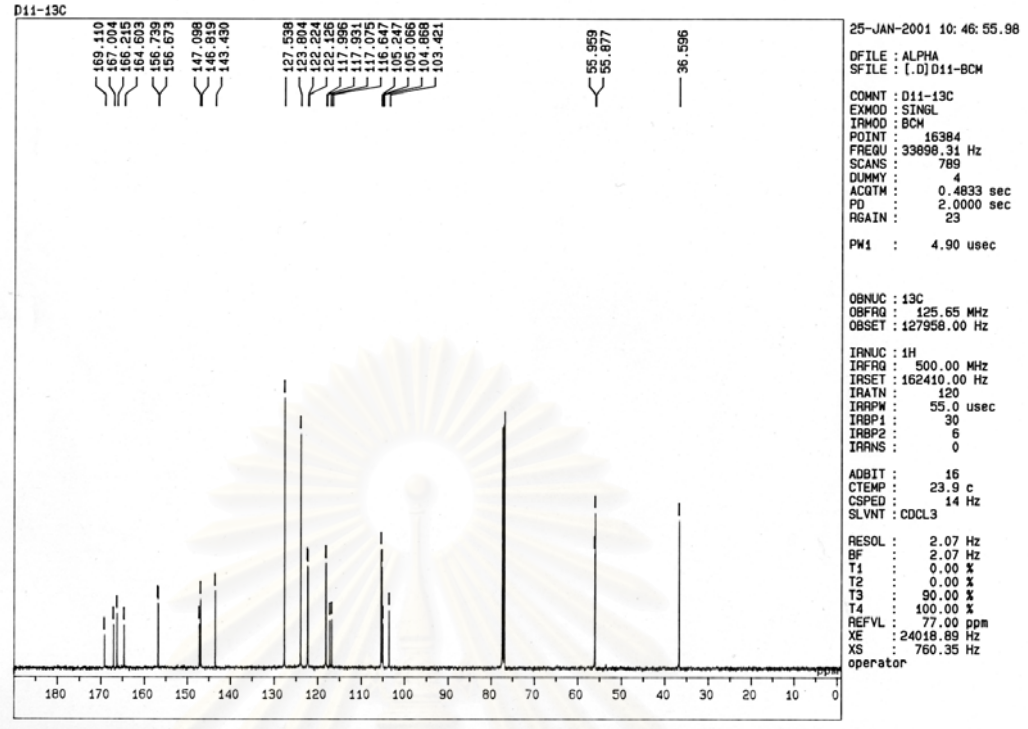


Fig 27 The ^{13}C-NMR spectrum of Compound **D26**

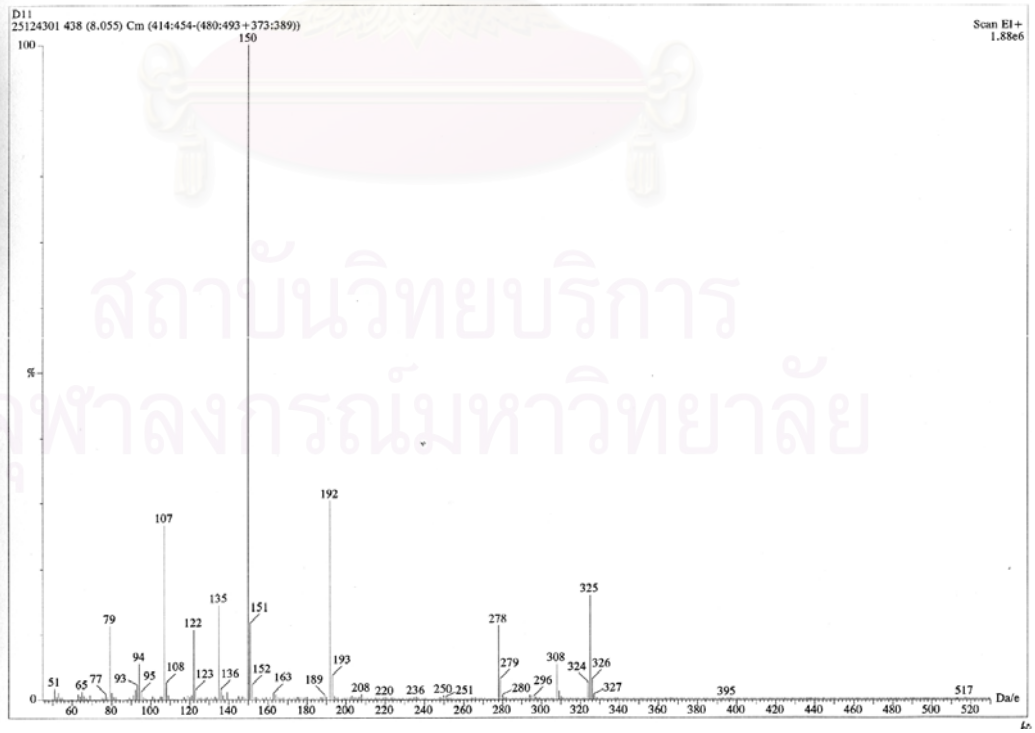


Fig 28 The mass spectrum of Compound **D26**

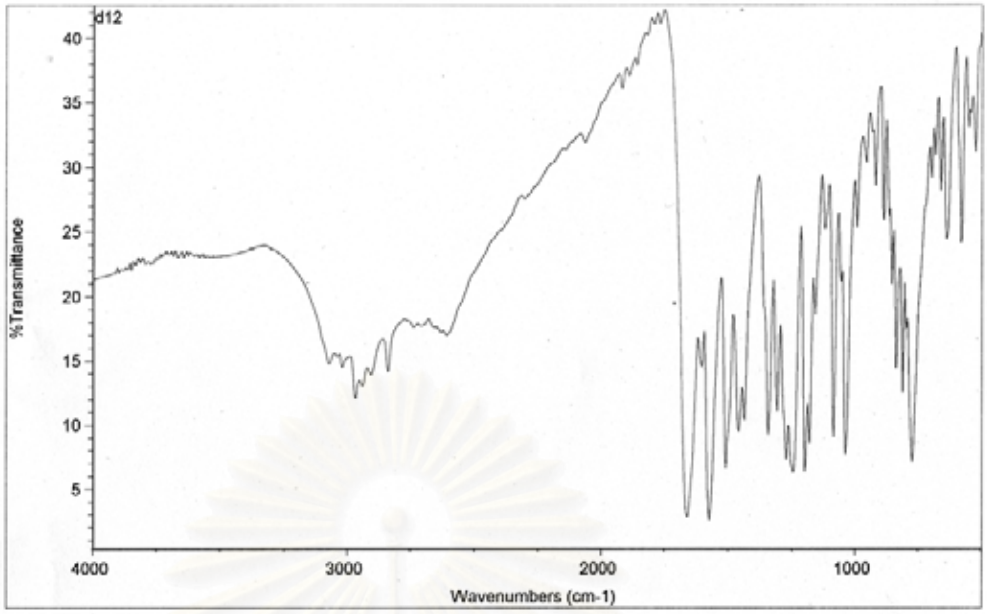


Fig 29 The FT-IR spectrum of Compound **D27**

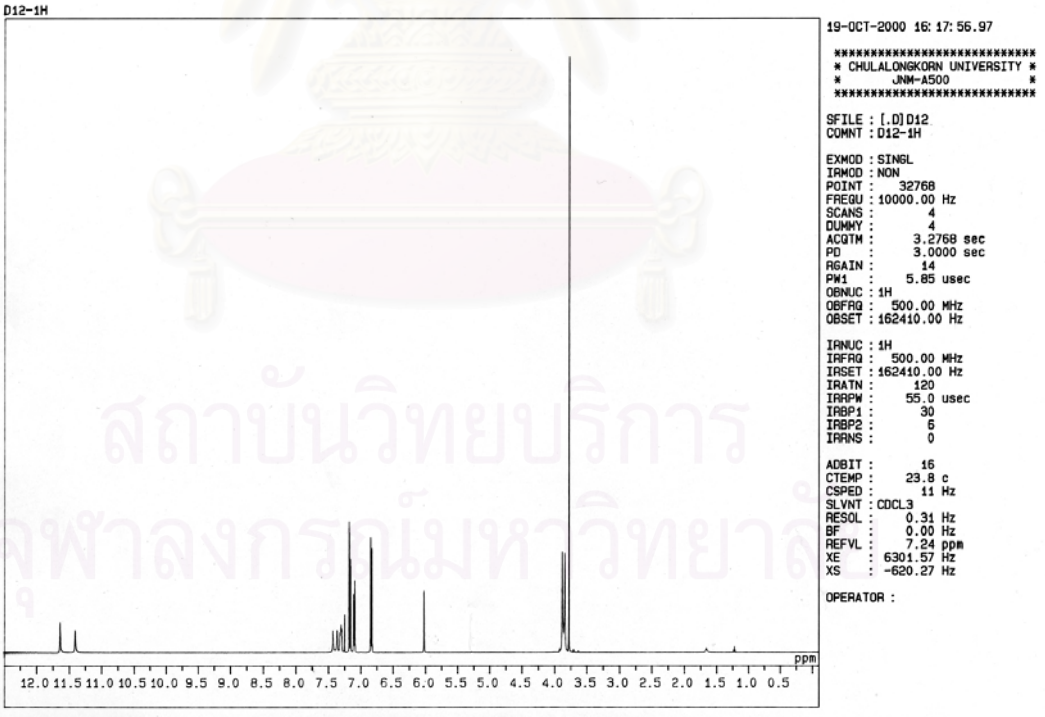


Fig 30 The ^{1}H-NMR spectrum of Compound **D27**

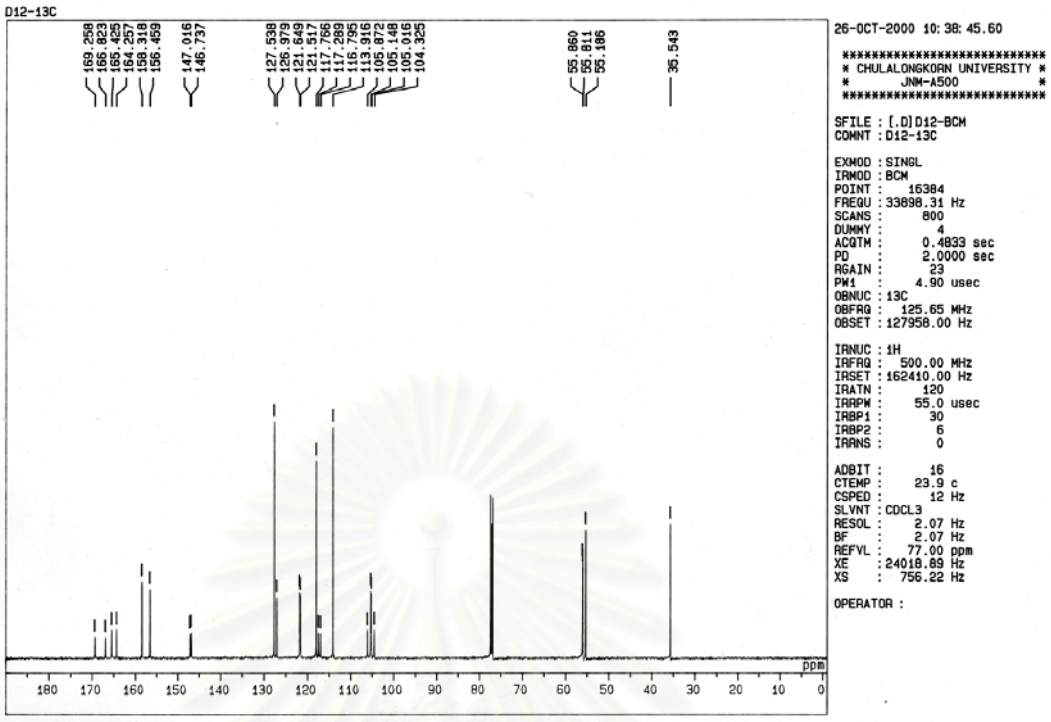


Fig 31 The ^{13}C-NMR spectrum of Compound **D27**

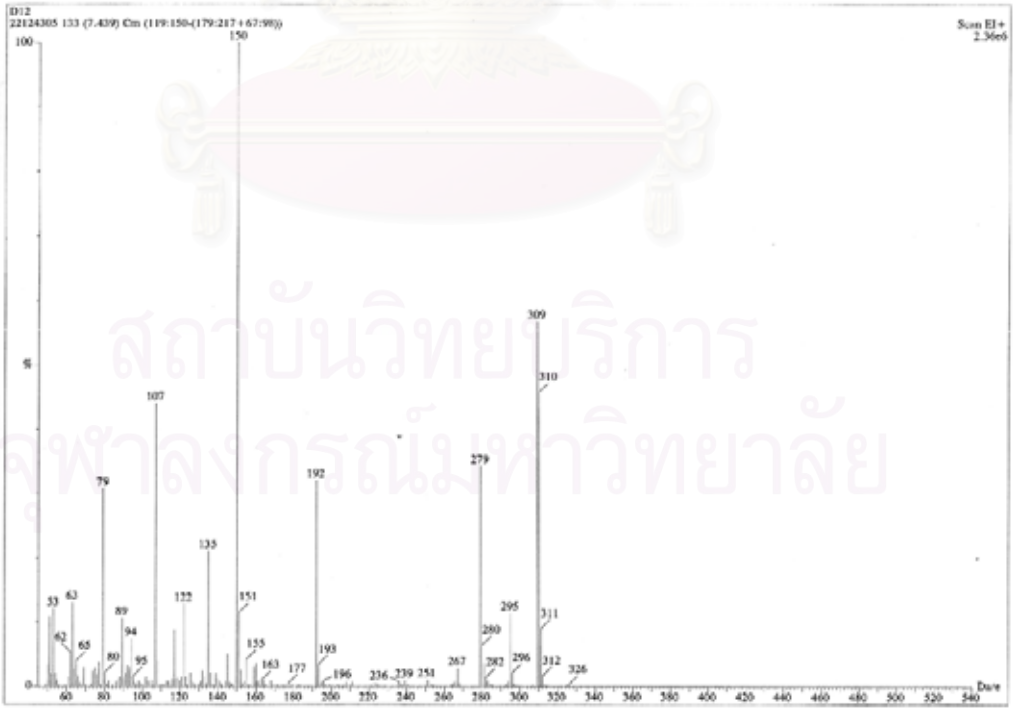


Fig 32 The mass spectrum of Compound **D27**

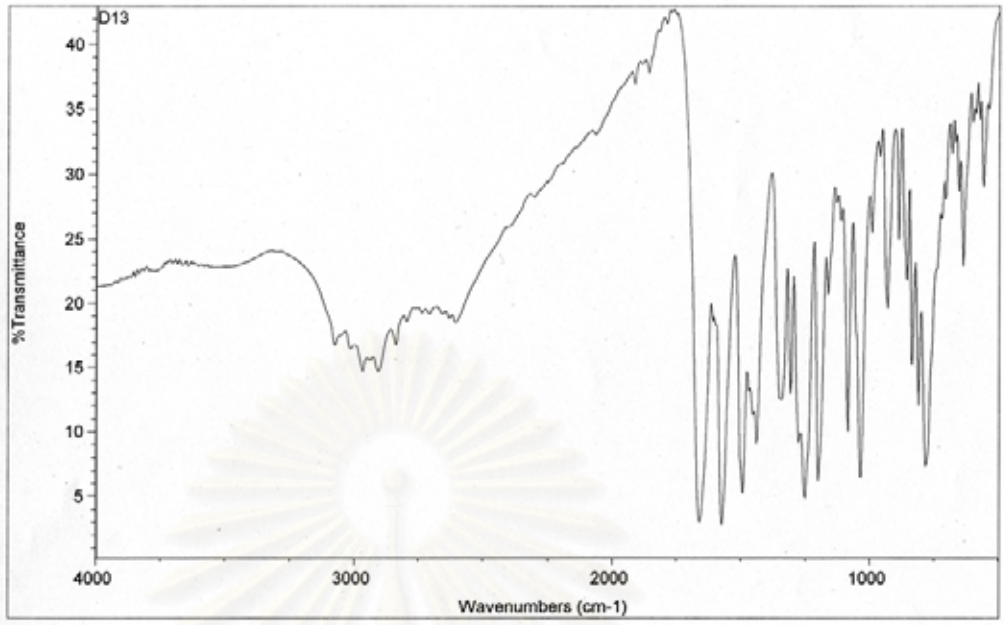


Fig 33 The FT-IR spectrum of Compound **D28**

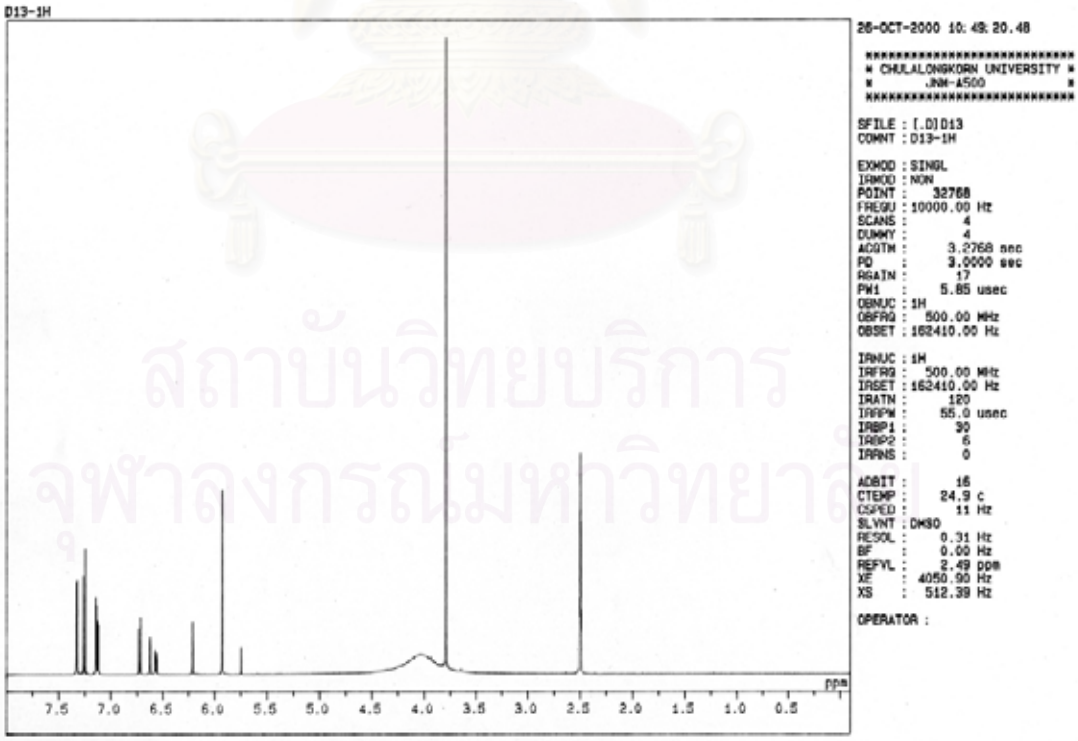


Fig 34 The ^{1}H-NMR spectrum of Compound **D28**

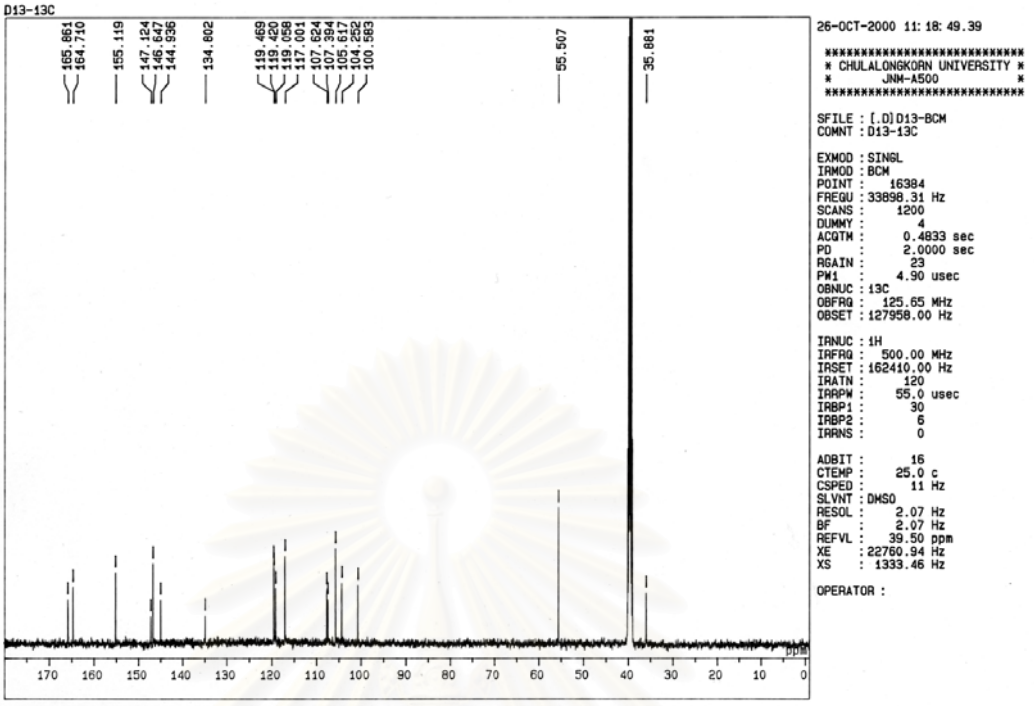


Fig 35 The ^{13}C-NMR spectrum of Compound **D28**

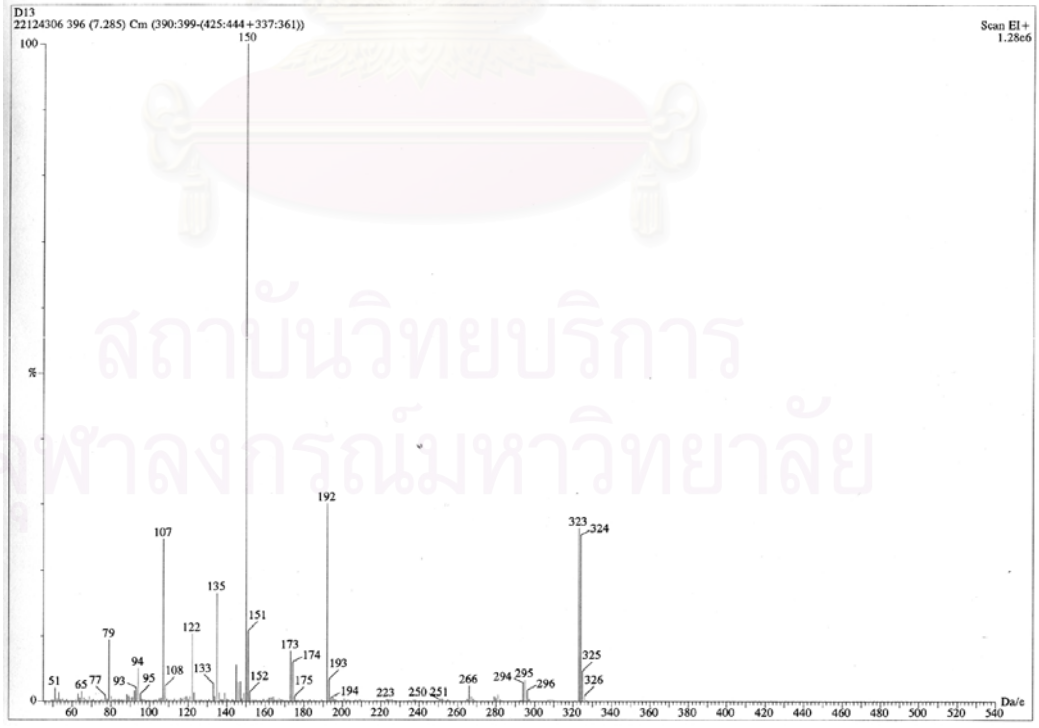


Fig 36 The mass spectrum of Compound **D28**

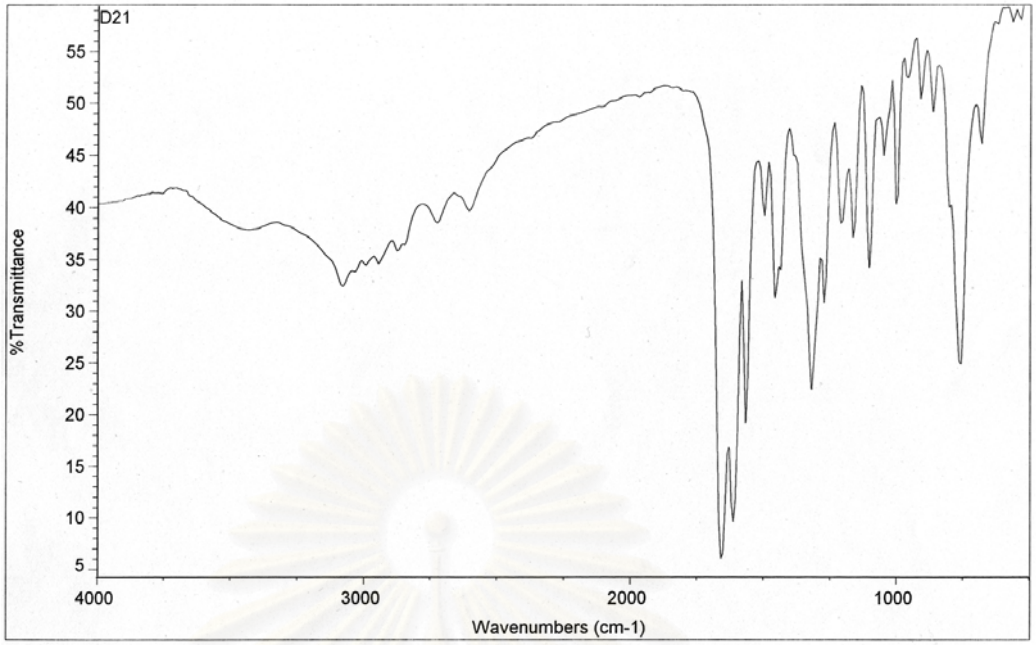


Fig 37 The FT-IR spectrum of Compound **D32**

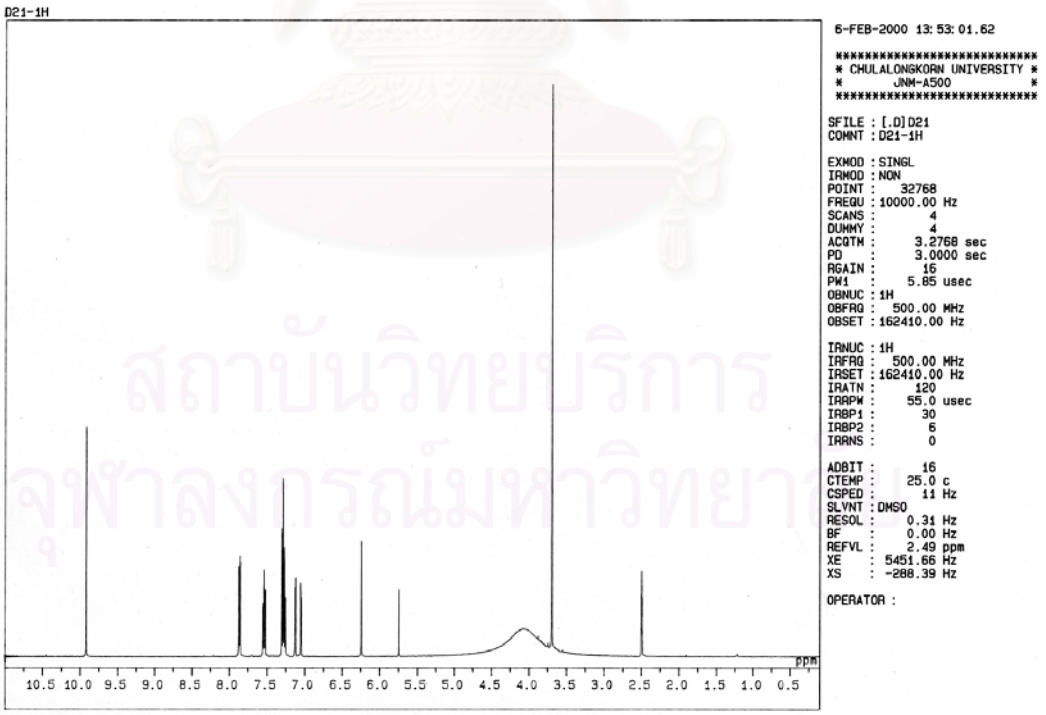


Fig 38 The ^{1}H-NMR spectrum of Compound **D32**

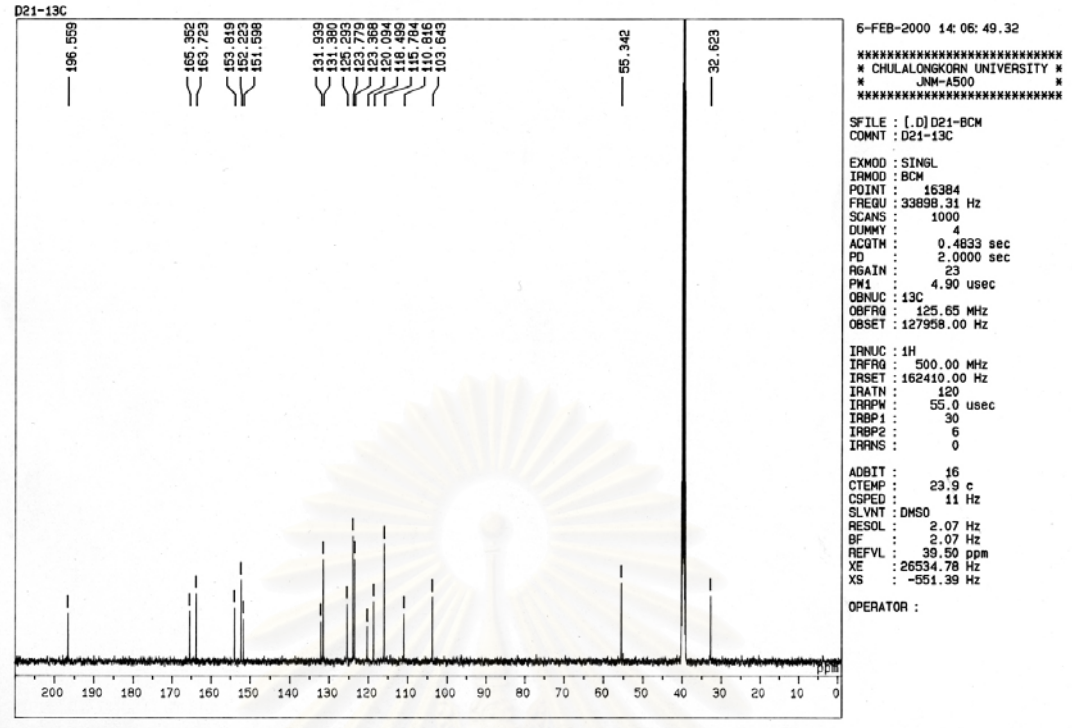


Fig 39 The ^{13}C-NMR spectrum of Compound **D32**

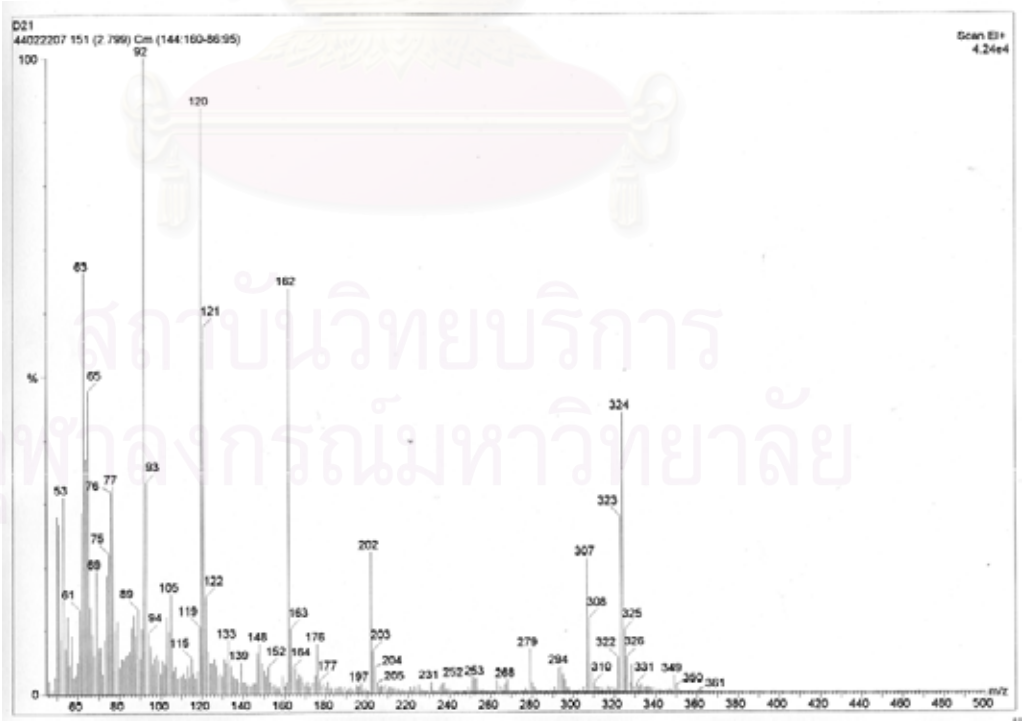


Fig 40 The mass spectrum of Compound **D32**

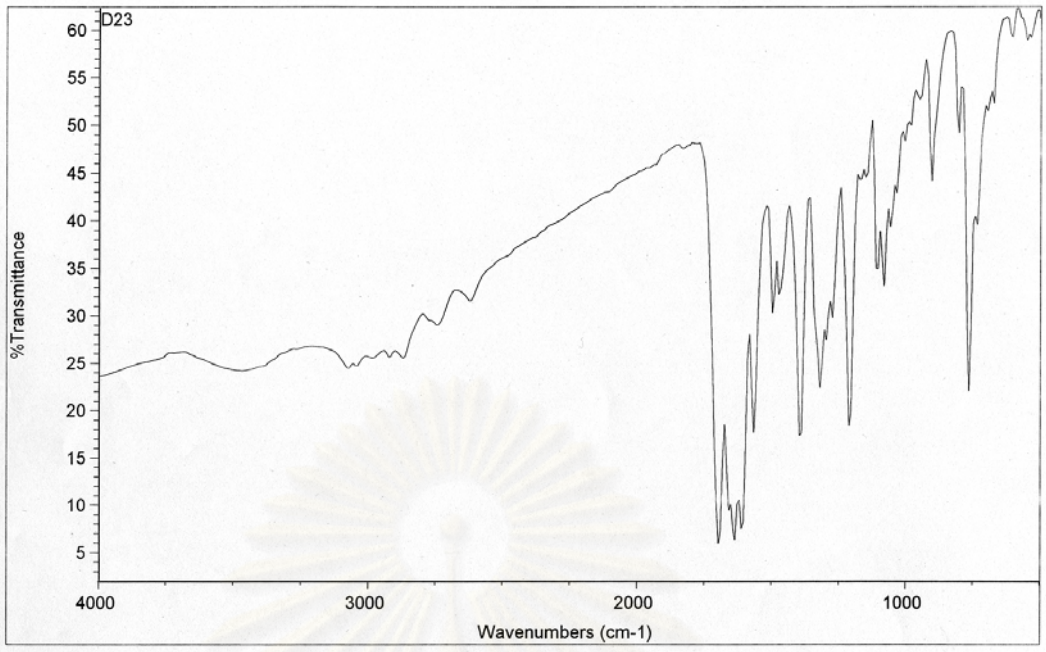


Fig 41 The FT-IR spectrum of Compound **D34**

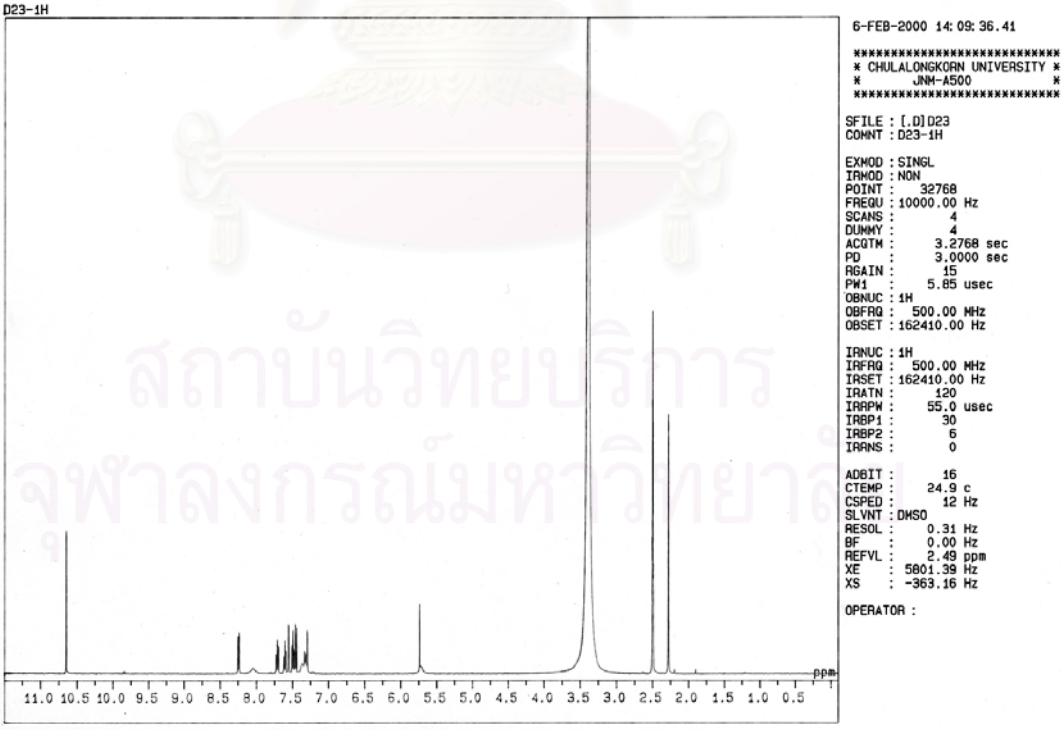


Fig 42 The ^{1}H-NMR spectrum of Compound **D34**

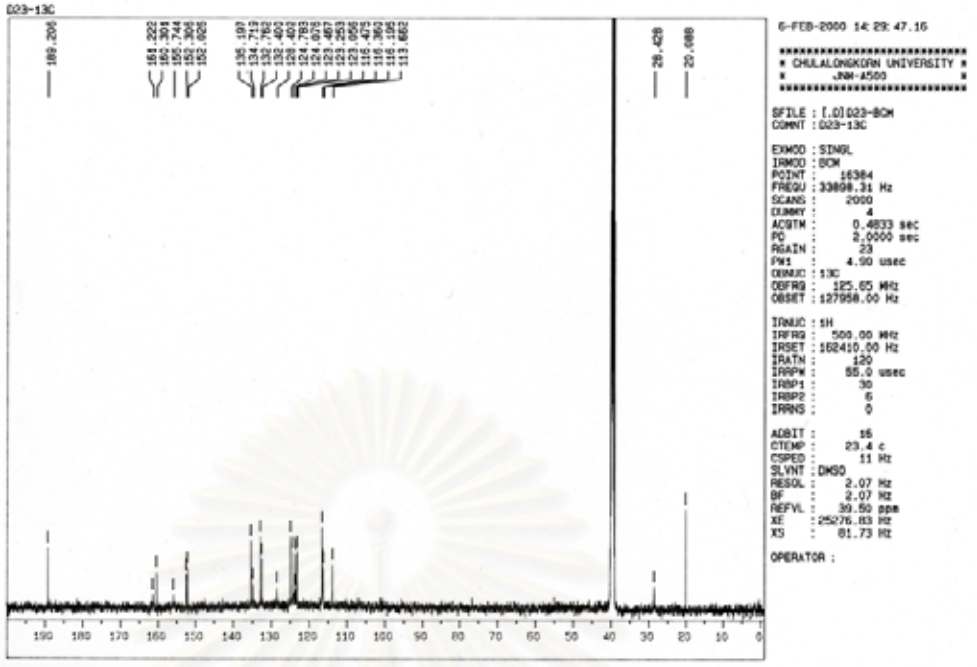


Fig 43 The ^{13}C-NMR spectrum of Compound **D34**

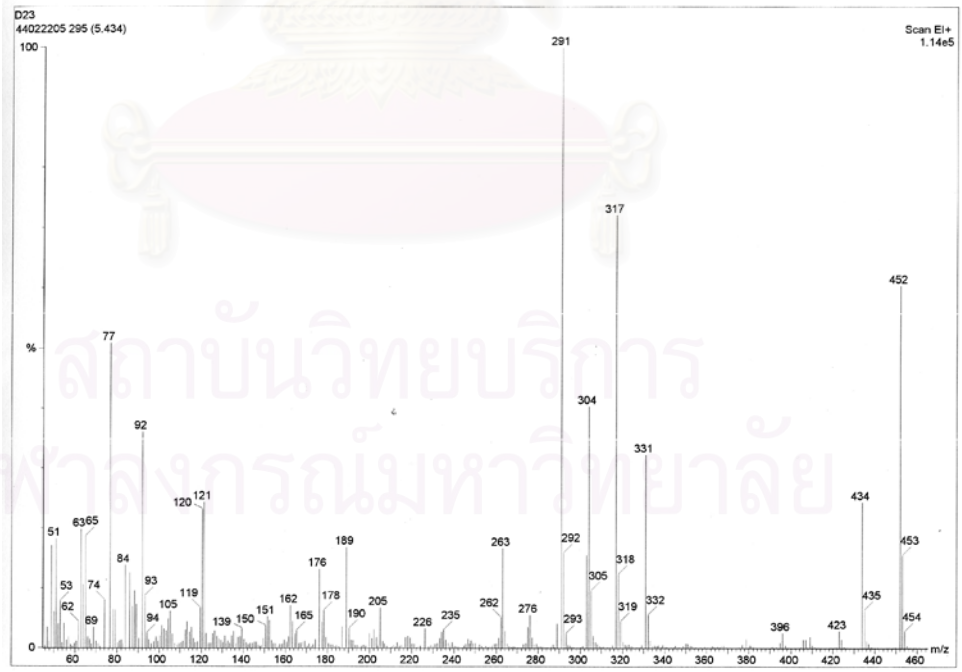


Fig 44 The mass spectrum of Compound **D34**

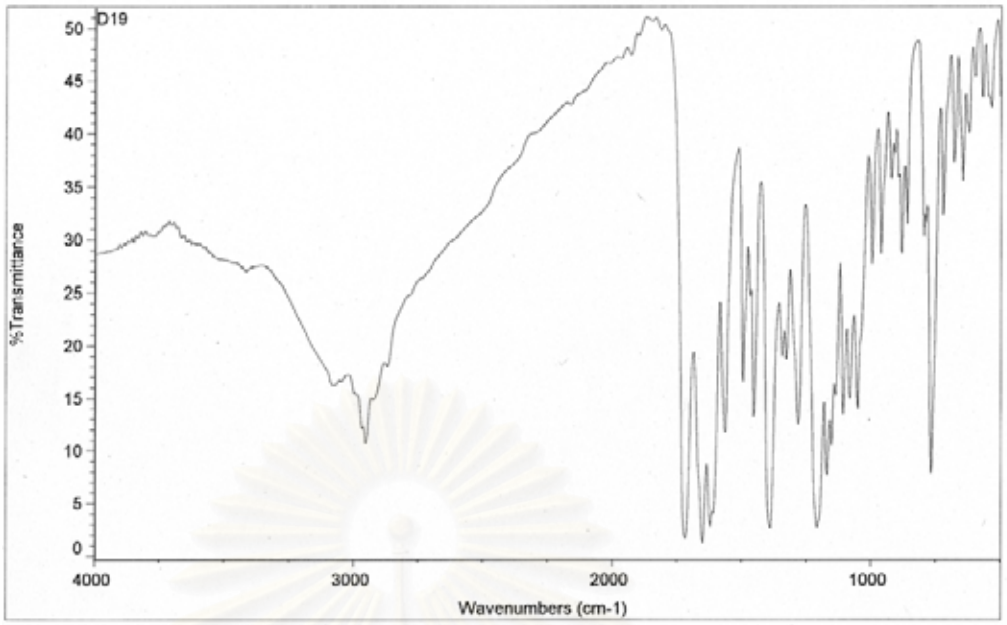


Fig 45 The FT-IR spectrum of Compound **D35**

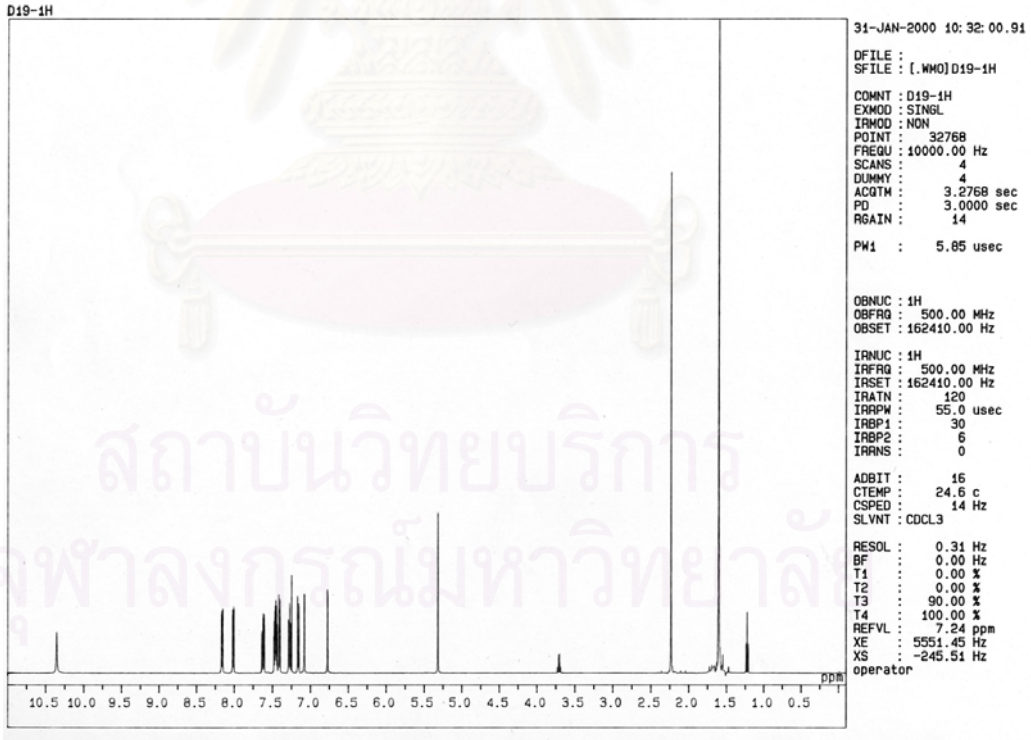


Fig 46 The ^{1}H-NMR spectrum of Compound **D35**

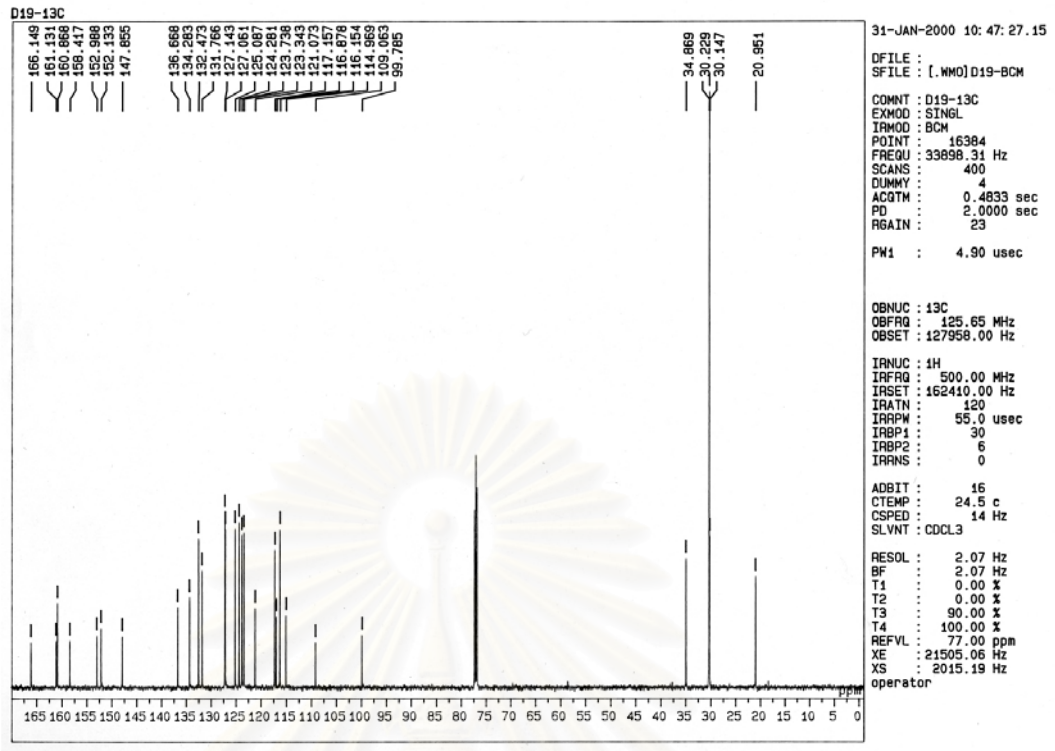


Fig 47 The ^{13}C-NMR spectrum of Compound **D35**

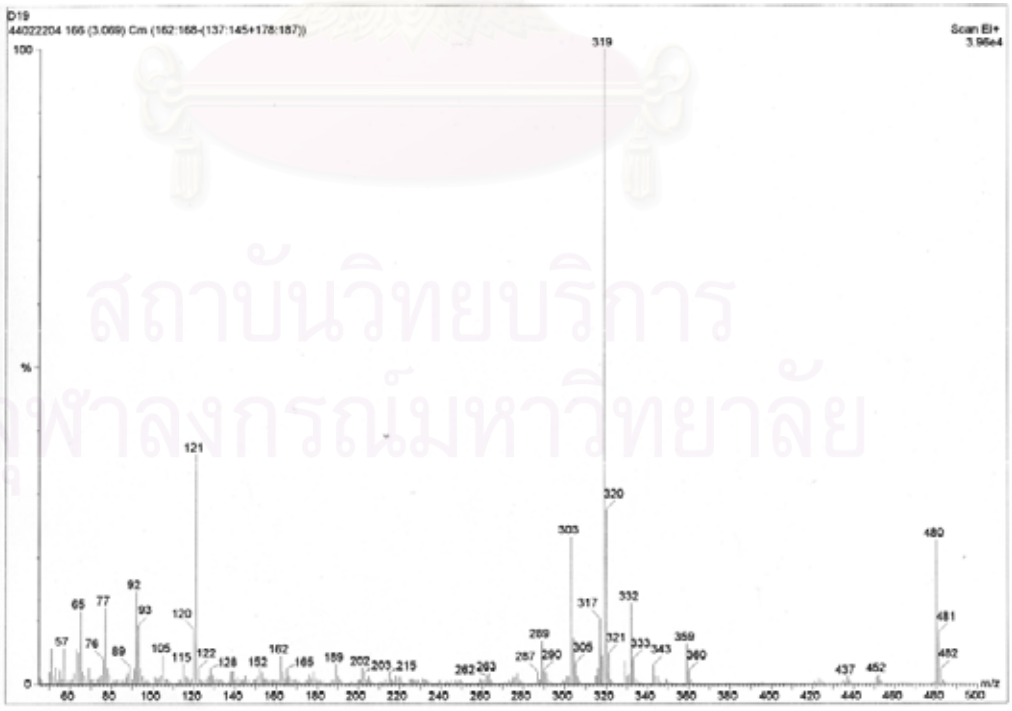


Fig 48 The mass spectrum of Compound **D35**

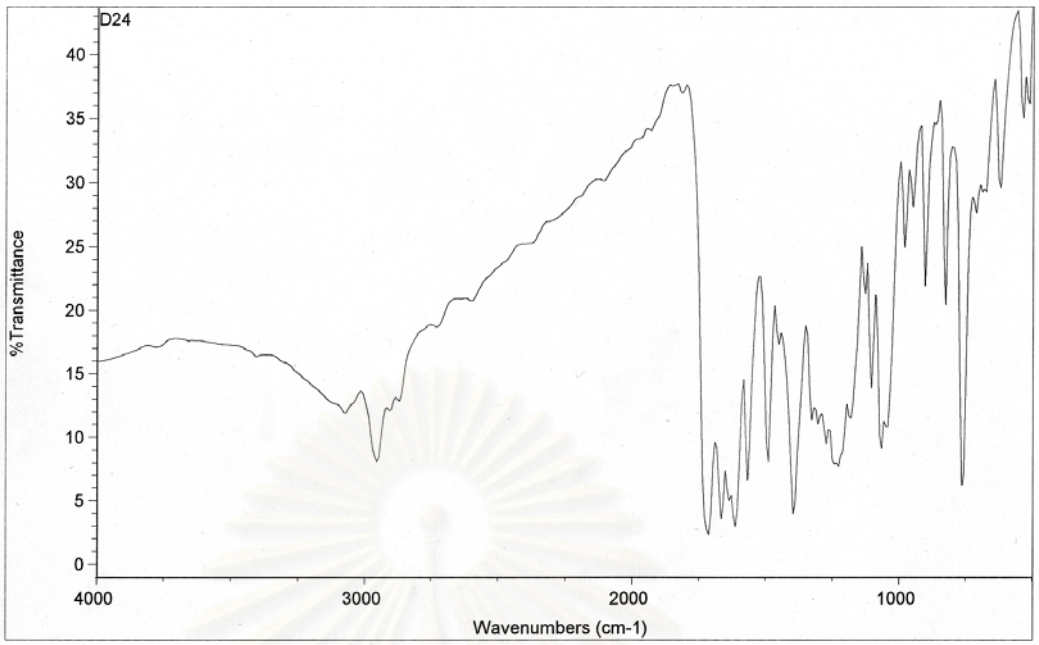


Fig 49 The FT-IR spectrum of Compound **D36**

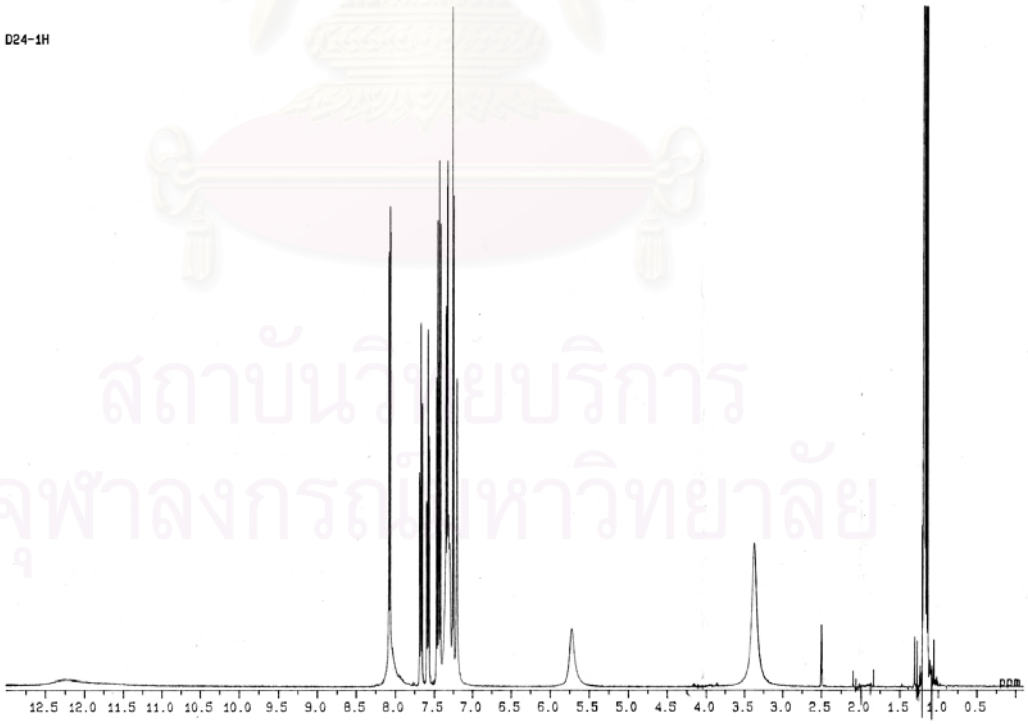


Fig 50 The ^{1}H-NMR spectrum of Compound **D36**

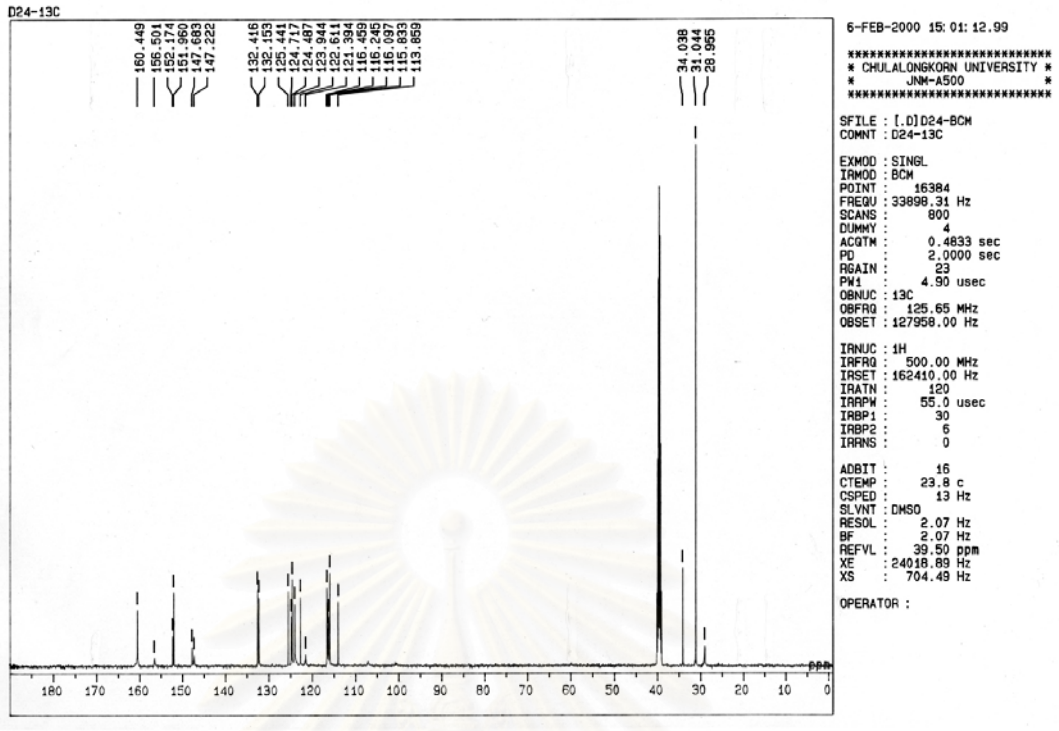


Fig 51 The ^{13}C-NMR spectrum of Compound **D36**

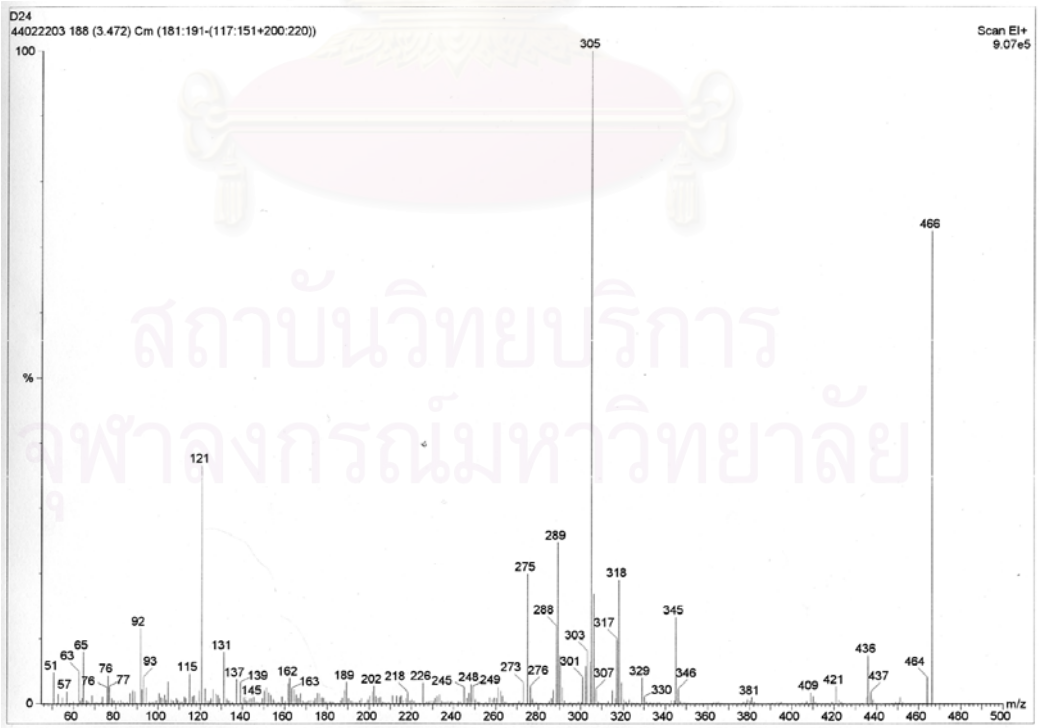


Fig 52 The mass spectrum of Compound **D36**

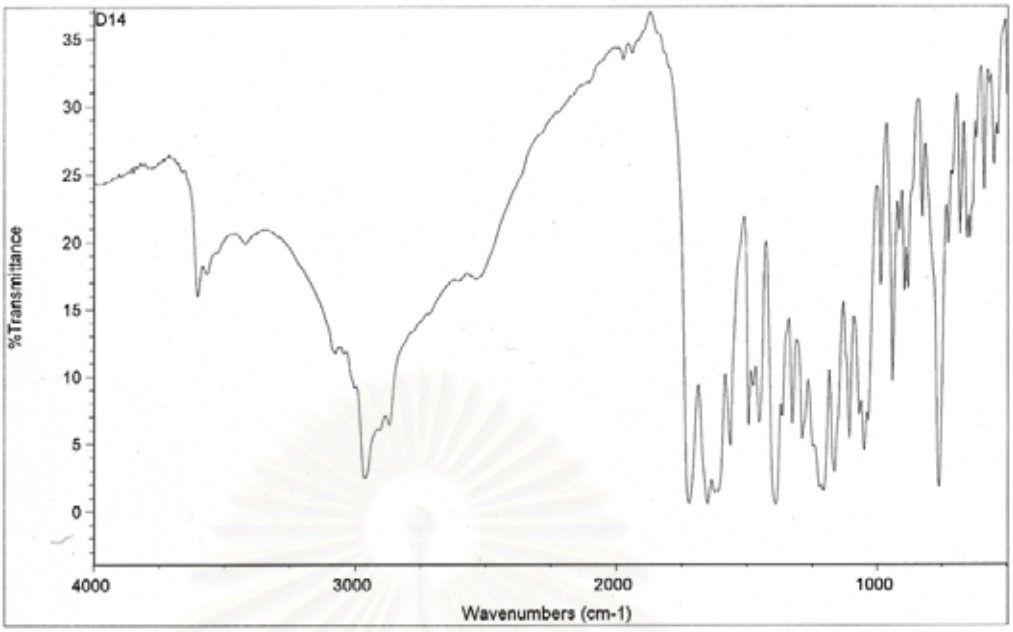


Fig 53 The FT-IR spectrum of Compound **D37**

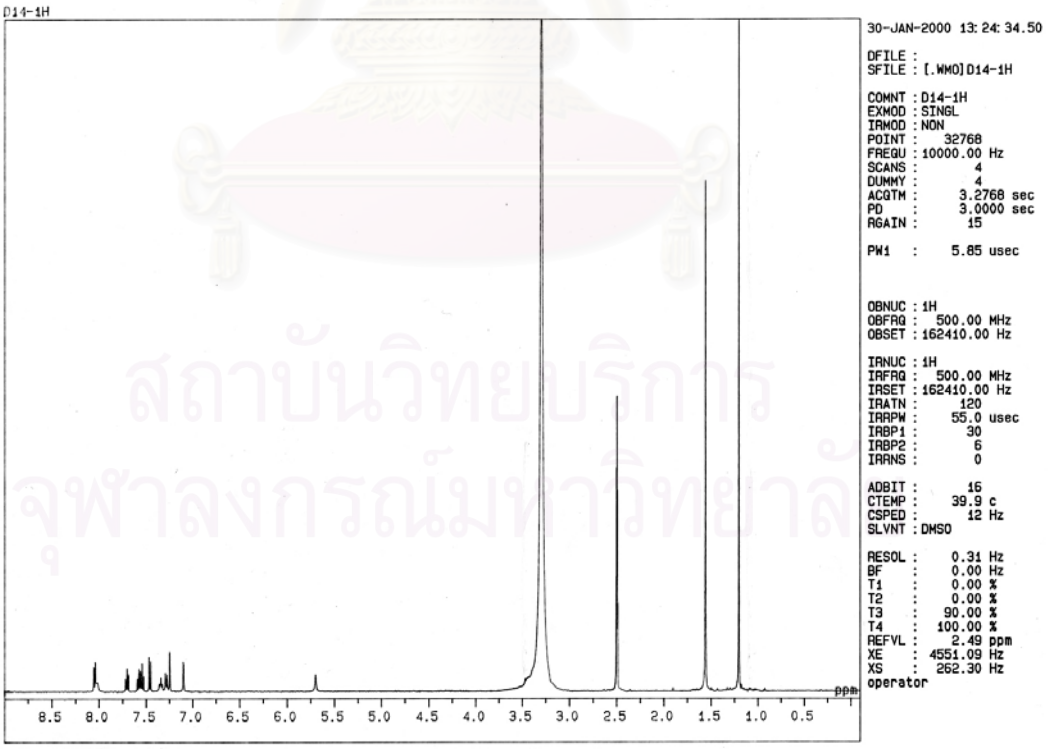


Fig 54 The ^{1}H-NMR spectrum of Compound **D37**

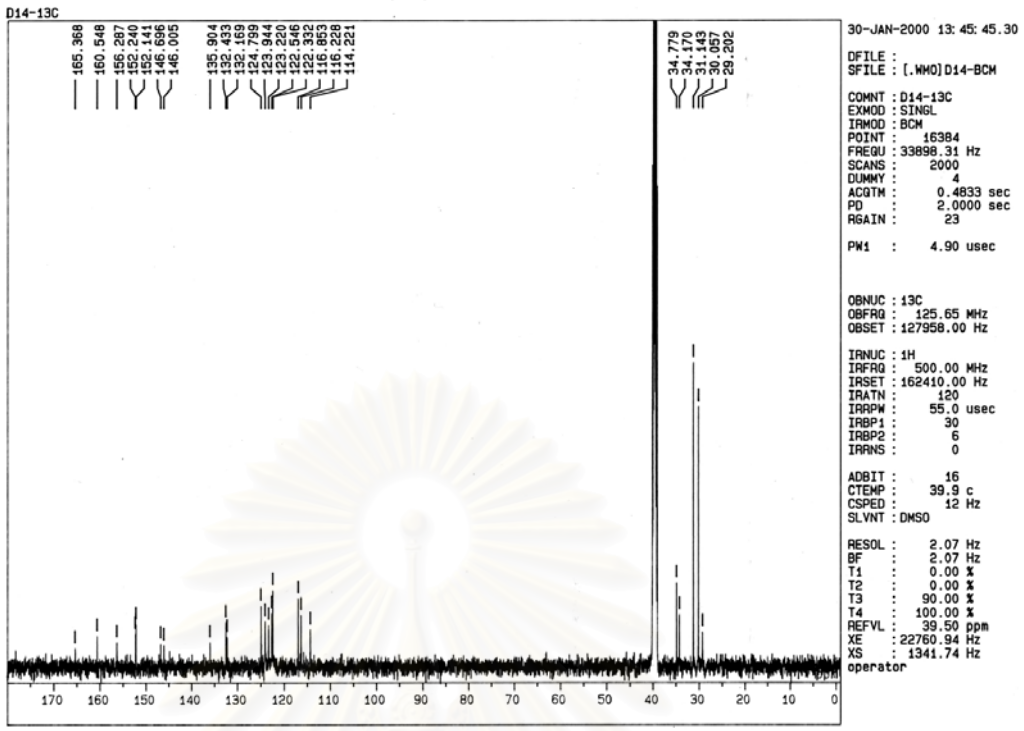


Fig 55 The ^{13}C-NMR spectrum of Compound **D37**

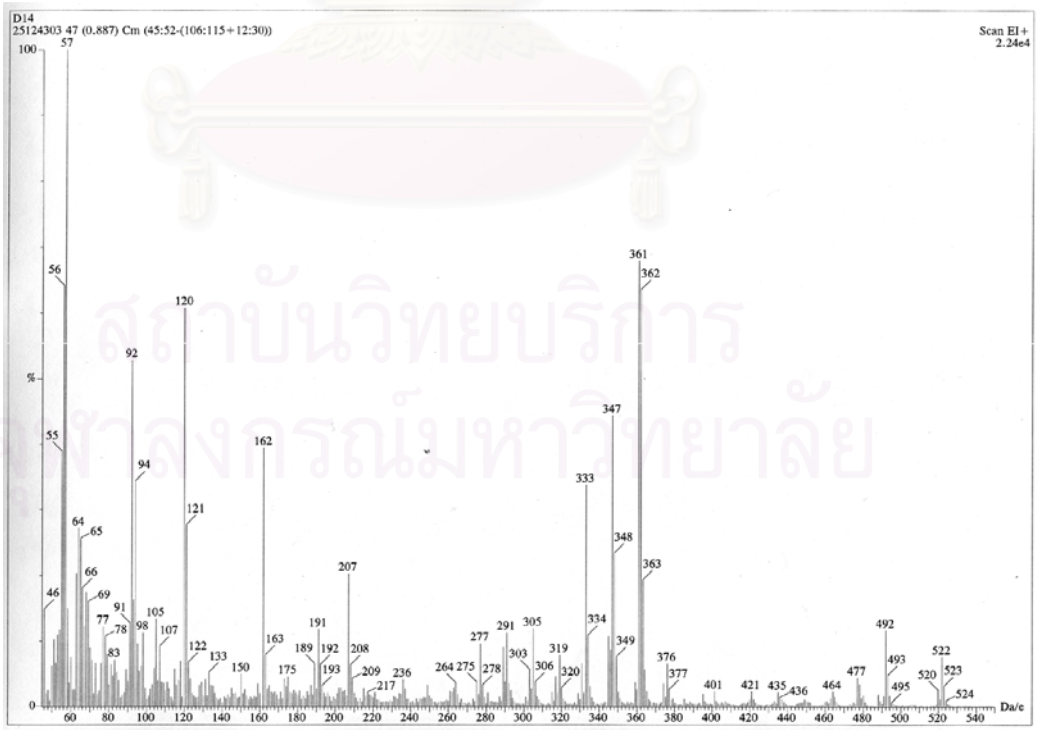


Fig 56 The mass spectrum of Compound **D37**

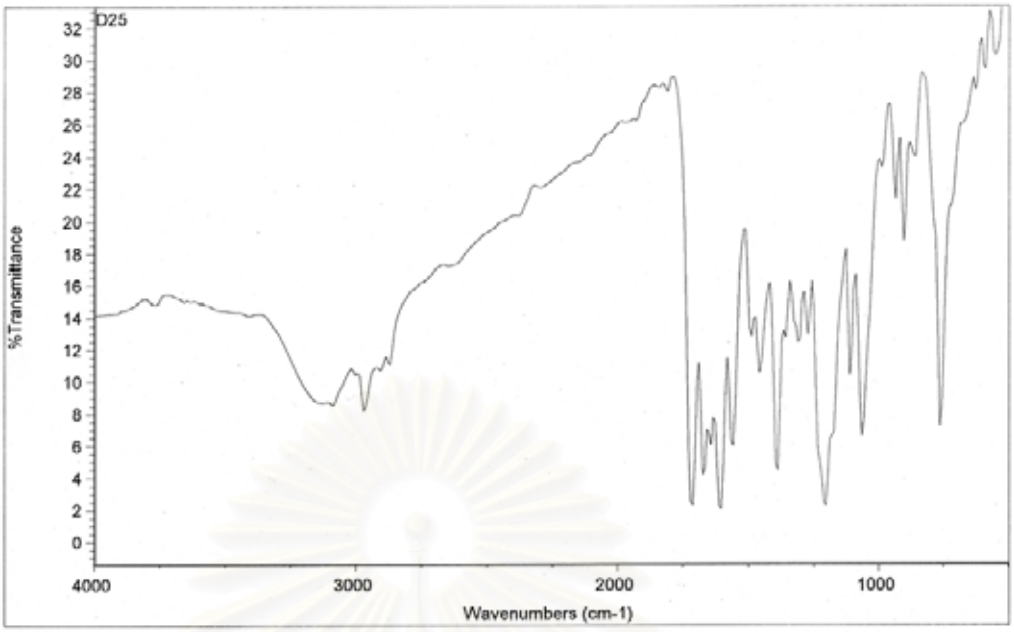


Fig 57 The FT-IR spectrum of Compound **D38**

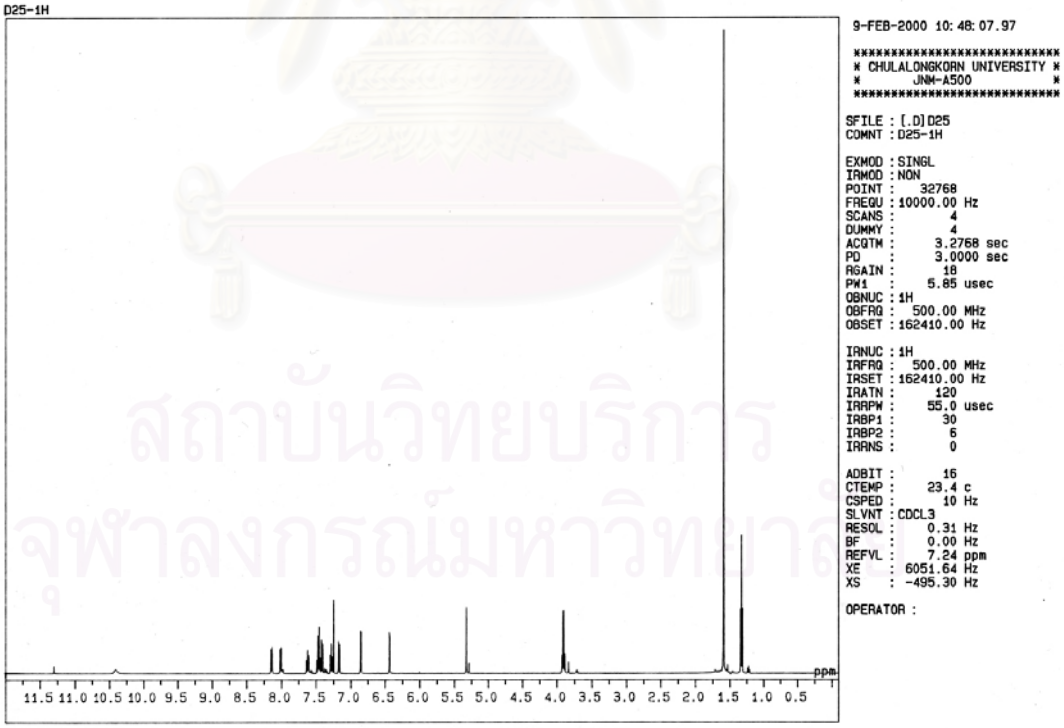


Fig 58 The ^{1}H-NMR spectrum of Compound **D38**

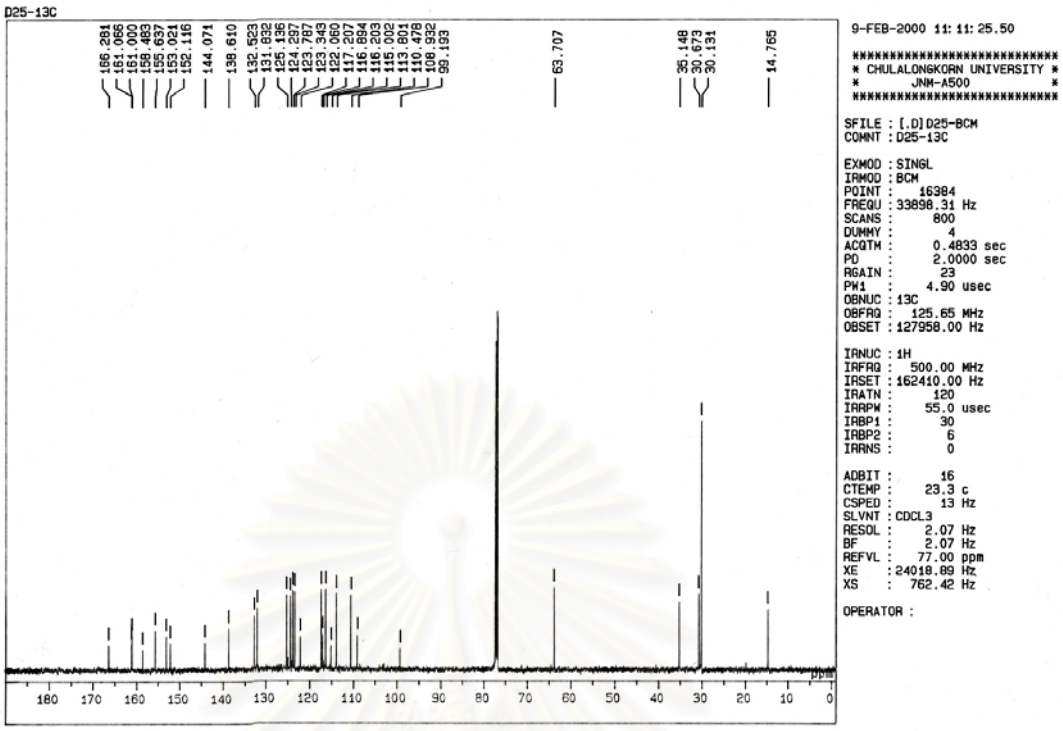


Fig 59 The ^{13}C-NMR spectrum of Compound **D38**

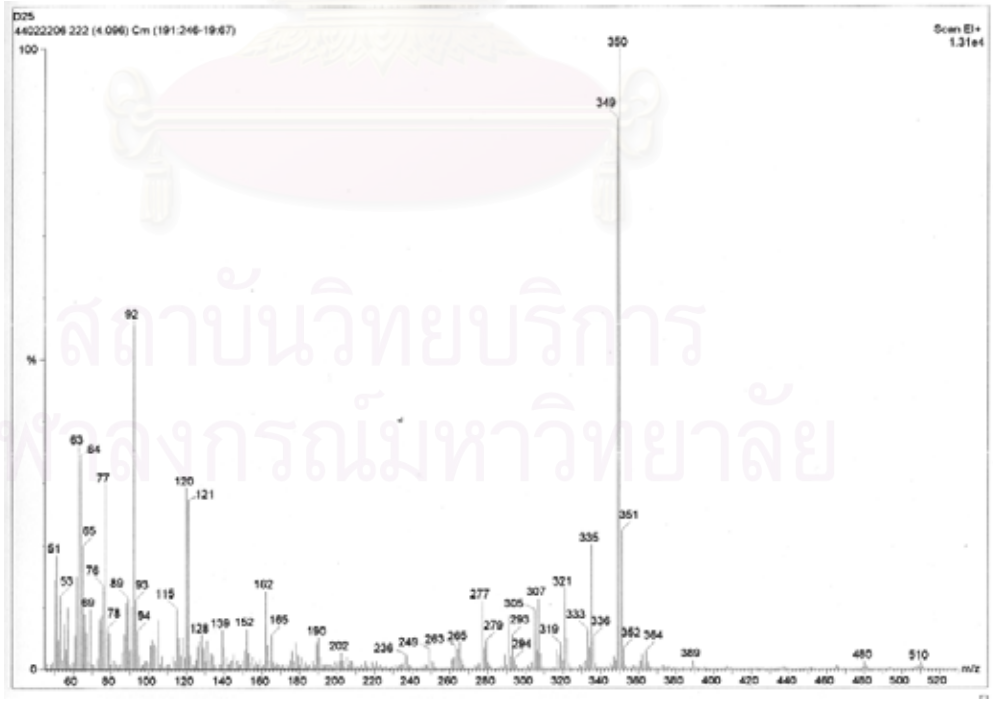


Fig 60 The mass spectrum of Compound **D38**

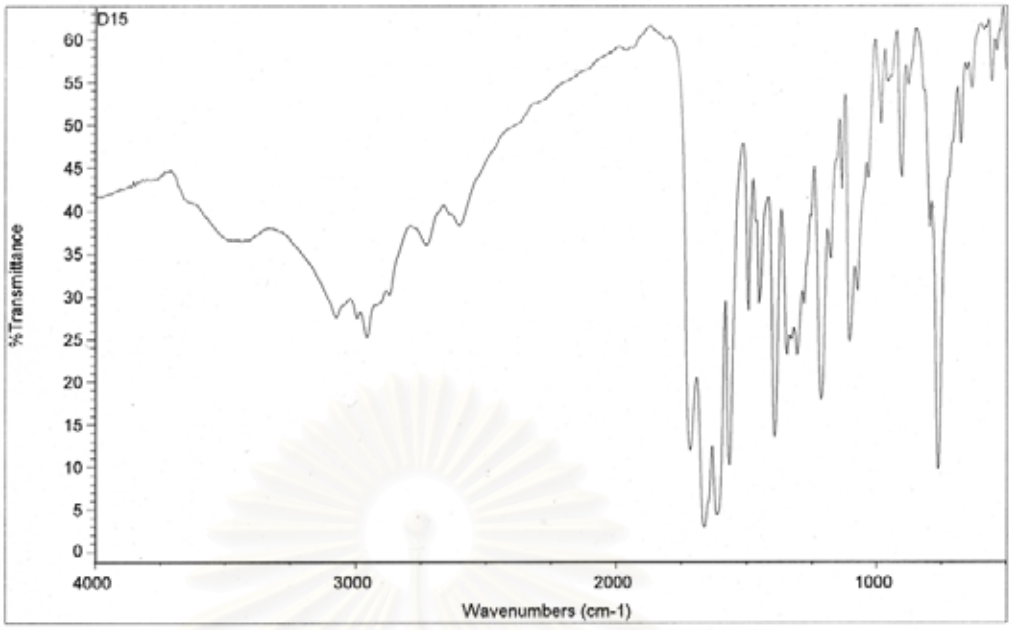


Fig 61 The FT-IR spectrum of Compound **D39**

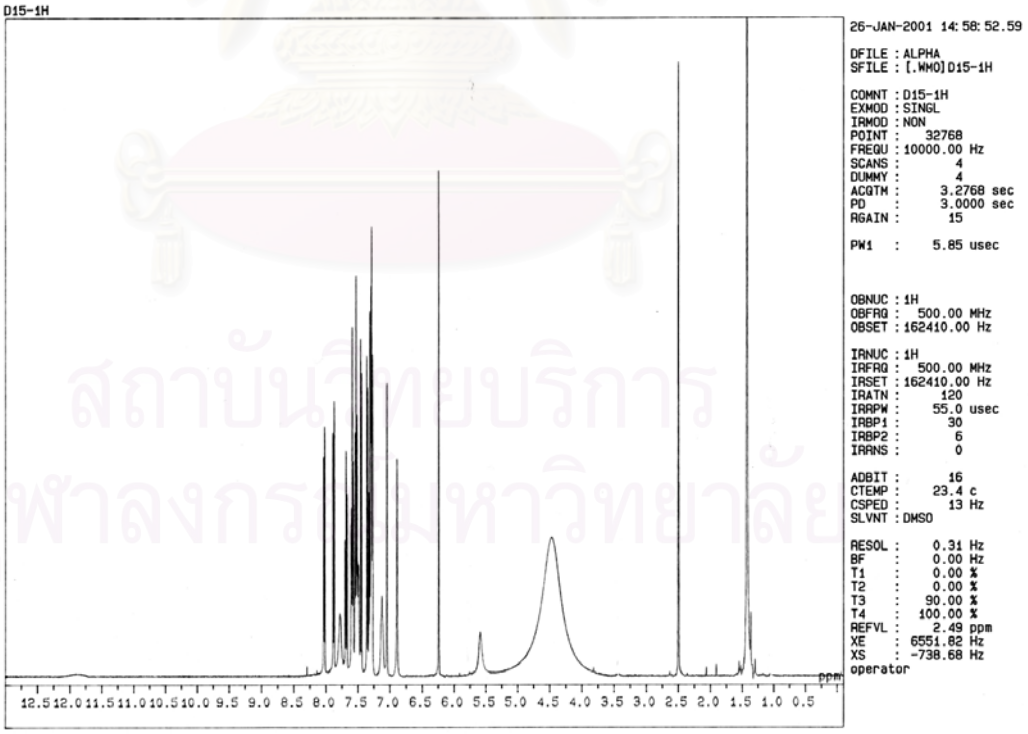


Fig 62 The ^{1}H-NMR spectrum of Compound **D39**

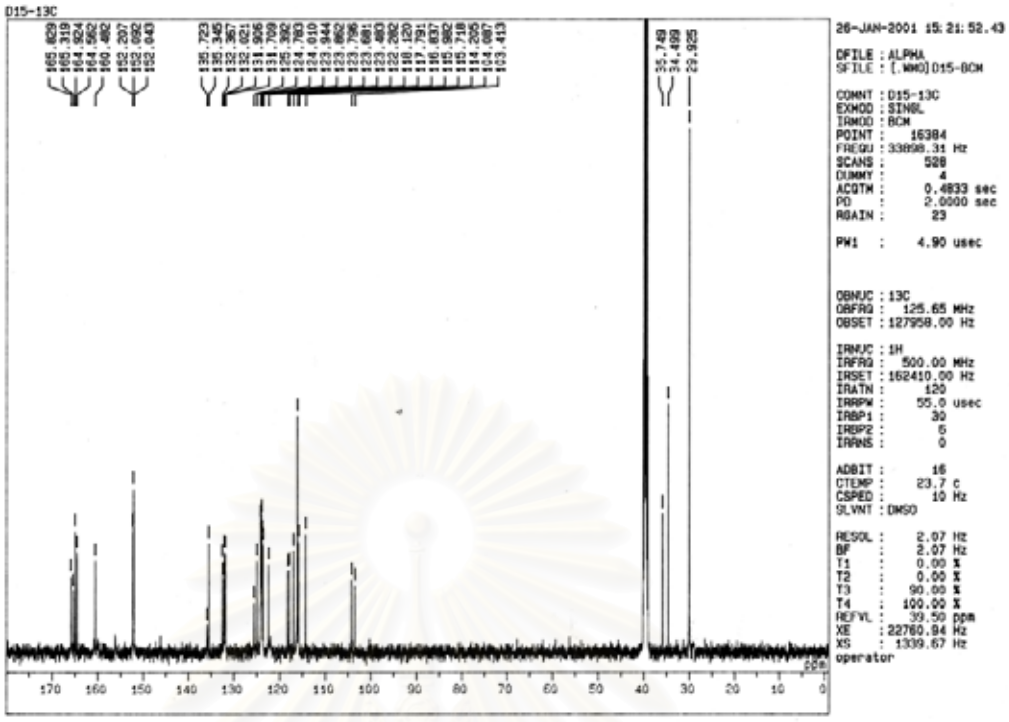


Fig 63 The ^{13}C-NMR spectrum of Compound **D39**

Fig 64 The mass spectrum of Compound **D39**

CURRICULUM VITAE

Miss Rouirush Worakijthamrongchai was born on September 1, 1976 in Supanburi, Thailand. She received a Bachelor Degree of Science in Chemistry at Chulalongkorn University in 1998. Since 1998, she has been a graduate student studying Organic Chemistry at Chulalongkorn University. During her studies towards the Master's degree, she was awarded a teaching assistant scholarship by the Faculty of Science during 1998-2000 and was supported by a research grant for her Master's thesis from the Graduate School, Chulalongkorn University.

Her present address is 128/303 Soi Ramkhamhaeng 24, Huamark, Bangkapi, Bangkok, Thailand 10240.