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PHYTOCHEMISTRY OF *PLOIARIUM ALTERNIFOLIUM* LEAVES

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การศึกษาทางพฤกษเคมีของใบชะมวงกวาง โดยใช้วิธีทางโครมาโทกราฟี สามารถแยกองค์ประกอบทางเคมีจากสิ่งสกัดในบิวทานอลได้สาร 4 ชนิด เป็นสารในกลุ่ม flavone glycosides คือ isoscutellarein 8-*O*-β-D-glucuronopyranoside methyl ester, hypolaetin 8-*O*-β-D-glucuronopyranoside methyl ester, isoscutellarein 8-*O*-β-D-glucuronopyranoside butyl ester และ hypolaetin 8-*O*-β-D-glucuronopyranoside butyl ester การพิสูจน์โครงสร้างทางเคมีของสารประกอบที่แยกได้นี้ อาศัยการวิเคราะห์สเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว

ภาควิชา เภสัชเวท
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ลายมือชื่อนิสิต ....นงกช พันธ์บูรณานนท์....
ลายมือชื่ออาจารย์ที่ปรึกษา ....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม ....

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Phytochemical study of the butanol extract of *Ploiarium alternifolium* leaves by chromatography led to the isolation of four flavone glycosides. These compounds are isoscutellarein 8-*O*-β-D-glucuronopyranoside methyl ester, hypolaetin 8-*O*-β-D-glucuronopyranoside methyl ester, isoscutellarein 8-*O*-β-D-glucuronopyranoside butyl ester, and hypolaetin 8-*O*-β-D-glucuronopyranoside butyl ester. The structures of these compounds were determined by analyses of the UV, IR, MS, NMR spectral data, as well as comparison with previously reported data.

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LIST OF ABBREVIATIONS

$[\alpha]^{25}_{D}$ = Specific rotation at 25°and sodium D line (589 nm)
δ = Chemical shift
λ_{max} = Wavelength at maximum absorption
ν_{max} = Wave number at maximum absorption
ε = Molar absorptivity
br d = Broad doublet
br s = Broad singlet
cm = Centimeter
°C = Degree Celsius
$CHCl_3$ = Chloroform
^{13}C NMR = Carbon-13 nuclear magnetic resonance
COSY = Correlation spectroscopy
d = Doublet
dd = Doublet of doublets
DEPT = Distortionless Enhancement by Polarization Transfer
DMSO-d_6 = Deuterated dimethylsulfoxide
ESI-TOF MS = Electrospray Ionization Time of Flight Mass spectrum
g = Gram
μg = Microgram
^{1}H NMR = Proton nuclear magnetic resonance
HMBC = ^{1}H-detected Heteronuclear Multiple Bond Coherence
HMQC= ^{1}H-detected Heteronuclear Multiple Quantum Coherence
Hz = Hertz
IC_{50} = Inhibitory concentration at 50%
IR = Infrared
J = Coupling constant
kg = Kilogram
L = Liter
m = Multiplet
mg = Milligram

ml = Milliliter
min = Minute
mult. = Multiplicity
M^+ = Molecular ion
MeOH = Methanol
MHz = Megahertz
$[M+H]^+$ = Protonated molecular ion
m/z = Mass to charge ratio
MS = Mass spectrometry
nm = Nanometer
NMR = Nuclear magnetic resonance
ppm = part per million
quin = Quintet
r.p.m. = Round per minute
s = Singlet
t = Triplet
TLC = Thin layer chromatography
UV = Ultraviolet

CHAPTER I

INTRODUCTION

Ploiarium alternifolium (Vahl) Melchior is a tree in the family Guttiferae (Bonnetiaceae), distributed in Cambodia, Malaysia, Indonesia and Thailand (Keng, 1972). *P. alternifolium* is the only species of this genus found in Thailand and can be found in lowland evergreen forest and peat swamp forest in Krabi, Satun, Songkhla, Surat Thani and Trang. It is locally known as Chamuang kwang (ชะมวงกวาง), Muang kwang (ม่วงกวาง), and Som Kwang (ส้มกวาง) (Smitinand, 1980).

Ploiarium alternifolium is a small tree, 8-10 meter high ; crown conical to cylindrical, with terminalia–like branching ; stem with stilt roots, sometimes up to 1.5–2 meter high; bark greyish-brown, narrowly and deeply ridged and fissured; inner bark red brown. *Leaves* simple, spirally arranged, crowded at the tip of the branches; blade lanceolate to oblong-obovate, 7-12 by 1.5-2.5 cm, glabrous; apex acute; base truncate to subcordate; secondary nerves inconspicious; petiole short. *Flowers* pinkish-white, 2.5-3 cm across; solitary or 2-3-flowered in short, axillary inflorescence. *Capsules* conical, 2 by 1 cm; basally dehiscing into 5 valves at maturity. Flowering from March to May (Phengklai and Niyomdham, 1991).

Previous phytochemical studies of the barks and branches of *P. alternifolium* have been reported (Bennett, Lee, and Lowrey, 1990; Bennett *et al*., 1991; 1992). However, the chemical constituents of its leaves have never been published. TLC examination of the butanol extract from the leaves of this plant showed several yellowish polar components. Therefore, it was interesting to investigate these compounds.

The main objectives in this investigation were as follows :

1. To extract and isolate pure compounds from the leaves of *P. alternifolium*.
2. To determine the chemical structures of the isolated compounds.

Fingure1 *Ploiarium alternifolium* (Vahl) Melchior

CHAPTER II

LITERATURE REVIEW

1. Taxonomy of *Ploiarium alternifolium*

Ploiarium alternifolium (Vahl) Melchior is a tree in the family Guttiferae (Bonnetiaceae) growing in peninsular Thailand. This plant possesses botanical characters and chemical constituents which are related to several plant families. In the past, it was classified as a member of the family Bonnetiaceae, in the order Theales (Hutchinson, 1959). Later, Bonnetiaceae was reclassified as subfamily Bonnetioideae of the family Theaceae (Mabberley, 1993). The different characteristic of this subfamily from other subfamilies was its convolute corolla while others were imbricate.

Currently, the genus *Ploiarium* has been included into the family Guttiferae (Mabberley, 1997) which composes of 3 subfamilies and 13 tribes.

The key to the subfamilies is as follows:

1. Leaves opposite, styles 3 or 5 ……………………………..Hypericoideae

1. Leaves spiral with 1, 3 or 5 styles or leaves opposite with 1, 2, 4 or 6 styles

    2. Flowers bisexual, fruit capsule ………………………...Bonnetioideae

    2. Flowers unisexual, bisexual, fruit not a capsule ……… Calophylloideae

I Subfamily Hypericoideae (3 tribes)

1.1 Tribe Cratoxyleae : *Cratoxylum*
1.2 Tribe Hypericeae : *Hypericum*
1.3 Tribe Vismieae : *Vismia*, *Psorospermum*

II Subfamily Bonnetioideae (3 tribes)
2.1 Tribe Bonnetieae : *Bonnetia*, *Ploiarium*
2.2 Tribe Kielmeyereae : *Kielmeyera*
2.3 Tribe Caraipeae : *Caraipa*

III Subfamily Calophylloideae (Clusioideae) (7 tribes)
3.1 Tribe Calophylleae : *Calophyllum*, *Mammea*, *Mesua*
3.2 Tribe Endodesmieae : *Endodesmia*
3.3 Tribe Allanblackieae : *Allanblackia*
3.4 Tribe Moronbeae : *Montrouziera*, *Pentadesma*, *Symphonia*
3.5 Tribe Garcinieae : *Garcinia*
3.6 Tribe Clusieae : *Clusia*
3.7 Tribe Tovomiteae : *Tovomita*, *Tovomitopsis*

According to these data, genus *Ploiarium* has been classified as a member of the subfamily Bonnetioideae of either the family Theaceae or Guttiferae, indicating the relationship of this genus with both families.

2. Chemical constituents of Guttiferae

Various compounds have been isolated from many genera of the family Guttiferae. These compounds were xanthones, flavonoids, benzophenones, coumarins, anthraquinones, terpenoids, and phloroglucinol derivatives.

Benzophenones were isolated from plant in subfamily Calophylloideae particularly genera *Garcinia* [*G. subelliptica* (Minami *et al*., 1998)] and *Clusia* [*C. ellipticifolia* (Olivares, Gonzalez, and Monache, 1994)] of the family Guttiferae.

Coumarins were isolated from genus *Kielmeyera* [*K. argentea* (Cruz, Santos *et al*., 1998), *K. reticulata* (Cruz, Moreira, *et al*., 1998)] of subfamily Bonnetioideae , genus *Vismia* [*V. guianensis* (Seo, *et al*., 2000)] of subfamily Hypericoideae and genus *Montrouziera* [*M. sphaeroidea* (Ito *et al*., 2000)] of subfamily Calophylloideae of the family Guttiferae.

Anthraquinones were found in genus *Ploiarium* [*P. alternifolium* (Bennett *et al*., 1991)] of subfamily Bonnetioideae and genera *Vismia* [*V. guineensis* (Seo *et al*., 2000)] of subfamily Hypericoideae and *Hypericum* [H. perforatum (Southwell and campbell, 1991)] of the family Guttiferae.

Triterpenoids were found in genus *Cratoxylum* [*C. cochinchinense* (Nguyen and Harison, 1999)] of subfamily Hypericoideae and genera *Ploiarium* [*P. alternifolium* (Bennett *et al*., 1992)] and *Kielmeyera* [*K. coriacea* (Cortez *et al*., 1998)] of subfamily Bonnetioideae of the family Guttiferae.

Phloroglucinol derivatives were found genus *Hypericum* [*H. brasiliense, H. japonicum*, *H. uliginosum* (Rocha *et al.*, 1995)] of subfamily Hypericoideae of the family Guttiferae.

Several genera of family Guttiferae containing xanthones and flavonoids were found in Thailand. The distribution of these compounds is shown in table 1 and 2.

Table 1 Distribution of xanthones in Guttiferae.

Plant and chemical compound	Plant part	Activity	Reference
Bonnetia dinizii			
1,7–Dihydroxyxanthone **[1]**	Wood	ND	Oliveira *et al*., 1990
1,7,8–Trihydroxyxanthone **[2]**	Wood	ND	Oliveira *et al*., 1990
1,6,7,8–Tetrahydroxy xanthone **[3]**	Wood	ND	Oliveira *et al*., 1990
1,2,3,5–Tetrahydroxy xanthone **[4]**	Wood	ND	Oliveira *et al*., 1990
1,3–Dihydroxy–2,5–dimethoxyxanthone **[5]**	Wood	ND	Oliveira *et al*., 1990
Calophyllum apetalum			
Caloxanthone I **[6]**	Stem bark	ND	Iinuma *et al*., 1997
Caloxanthone J **[7]**	Stem bark	ND	Iinuma *et al*., 1997
Caloxanthone K **[8]**	Root	ND	Iinuma *et al*., 1997
1,3,6,8–Tetrahydroxy–2–methoxyxanthone **[9]**	Root	ND	Iinuma *et al*., 1997
Pyranojacareubin **[10]**	Stem bark	ND	Iinuma *et al*., 1997
1,3,5–Trihydroxy–2–methoxyxanthone **[11]**	Stem bark	ND	Iinuma *et al*., 1997
1,3,5–Trihydroxyxanthone **[12]**	Stem bark	ND	Iinuma *et al*., 1997
1,5–Dihydroxyxanthone **[13]**	Root	ND	Iinuma *et al*., 1997
3,8–Dihydroxy–1,2–dimethoxyxanthone **[14]**	Root	ND	Iinuma *et al*., 1997
1,3–Dihydroxy–2,5–dimethoxyxanthone **[15]**	Root	ND	Iinuma *et al*., 1997
1,3,7–Trihydroxyxanthone **[16]**	Root	ND	Iinuma *et al*., 1997
1,5,6–Trihydroxyxanthone	Root	ND	Iinuma *et al*., 1997

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Calophyllum inophyllum			
Caloxanthone A **[17]**	Root bark	ND	Iinuma *et al*., 1994
Caloxanthone B **[18]**	Root bark	ND	Iinuma *et al*., 1994
Caloxanthone D **[19]**	Root bark	ND	Iinuma *et al*., 1995
Caloxanthone E **[20]**	Root heartwood	ND	Iinuma *et al*., 1995
1,3,8–Trihydroxy–7–methoxyxanthone **[21]**	Root heartwood	ND	Iinuma *et al*., 1995
1,3–Dihydroxy–7,8–dimethoxyxanthone **[22]**	Root heartwood	ND	Iinuma *et al*., 1995
1,3,5–Trihydroxy–2–methoxyxanthone **[11]**	Root heartwood	ND	Iinuma *et al*., 1995
6–Hydroxy–1,5–dimethoxy xanthone **[23]**	Root heartwood	ND	Iinuma *et al*., 1995
Calophyllum moonii			
Dombakinaxanthone **[24]**	Root bark	ND	Dharmaratne and Wijesinghe, 1997
Calozeyloxanthone **[25]**	Root bark	ND	Dharmaratne and Wijesinghe, 1997
Calophyllum teysmannii var. *inophylloide*			
1,7– Dihydroxyxanthone **[1]**	Wood	ND	Kijjoa *et al*., 2000 a
1,2,8–Trimethoxyxanthone **[26]**	Wood	ND	Kijjoa *et al*., 2000 a
6–Hydroxy–1,3,5,7–tetra methoxyxanthone **[27]**	Wood	ND	Kijjoa *et al*., 2000 a
7–Hydroxy–1,2,8–trimethoxy xanthone **[28]**	Wood	ND	Kijjoa *et al*., 2000 b

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Calophyllum teysmannii var. *inophylloide*			
6–Hydroxy–1,2,5–trimethoxy xanthone **[29]**	Wood	ND	Kijjoa *et al.*, 2000 b
3,8–Dihydroxy–1,2,4–tri methoxyxanthone **[30]**	Wood	ND	Kijjoa *et al.*, 2000 b
3–Hydroxy–2,4–dimethoxy xanthone **[31]**	Wood	ND	Kijjoa *et al.*, 2000 b
1,7–Dihydroxy–3–methoxy xanthone (gentisin) **[32]**	Wood	ND	Kijjoa *et al.*, 2000 b
2–Carbomethoxy–6–methoxy xanthone [33]	Wood	ND	Kijjoa *et al.*, 2000 b
2–Hydroxyxanthone[**34**]	Wood	ND	Kijjoa *et al.*, 2000 b
Clusia insignis			
Clusone **[35]**	Flower	No antimicrobial	Ishiguro, Chaudhuri and Kubo, 1998
Cratoxylum cochinchinense			
Mangostin **[36]**	Bark	ND	Bennett *et al.*, 1993
β–Mangostin **[37]**	Bark	ND	Bennett *et al.*, 1993
Garcinone D **[38]**	Bark	ND	Bennett *et al.*, 1993
Tovophllin A **[39]**	Bark	ND	Bennett *et al.*, 1993
2–Geranyl–1,3,7–trihydroxy–4–(3,3–dimethylallyl)–xanthone [**40**]	Bark	ND	Bennett *et al.*, 1993
Cratoxylone **[41]**	Bark	ND	Bennett *et al.*, 1993
11–Hydroxy–1–isomangostin **[42]**	Bark	ND	Sia *et al.*, 1995
11–Hydroxy–3,6–di–*O*–methyl–1–isomangostin **[43]**	Bark	ND	Sia *et al.*, 1995

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Cratoxylum cochinchinense			
11–Acetoxy–3,6–di–*O*–methyl–1–isomangostin **[44]**	Bark	ND	Sia *et al*., 1995
1,3,5,6–Tetrahydroxy xanthone **[45]**	Bark	ND	Sia *et al*., 1995
Cratoxylum formosamum			
2,7–Dihydroxy–1,8–dimethoxyxanthone **[46]**	Root	ND	Iinuma *et al*., 1996 a
1,4,7–Trihydroxy–8–methoxyxanthone **[47]**	Root	ND	Iinuma *et al*., 1996 a
1,4,7–Trihydroxyxanthone **[48]**	Root	ND	Iinuma *et al*., 1996 a
1,7–Dihydroxy–4–methoxy xanthone **[49]**	Root	ND	Iinuma *et al*., 1996 a
1,7–Dihydroxyxanthone **[1]**	Root	ND	Iinuma *et al*., 1996 a
1,2,3,4,8–Pentamethoxy xanthone **[50]**	Root	ND	Iinuma *et al*., 1996 a
3,8–Dihydroxy-1,2-dimethoxyxanthone **[14]**	Root	ND	Iinuma *et al*., 1996 a
Cratoxylum maingayi			
1,7–Dihydroxyxanthone **[1]**	Wood	ND	Kijjoa *et al*., 1998
1,7–Dihydroxy–4–methoxy xanthone **[49]**	Wood	ND	Kijjoa *et al*., 1998
2,8–Dihydroxy–1–methoxy xanthone **[51]**	Wood	ND	Kijjoa *et al*., 1998
7–Hydroxy–1,2,3,8–tetra methoxyxanthone **[52]**	Wood	ND	Kijjoa *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Garcinia atroviridis			
Atroviridin **[53]**	Stem bark	ND	Kosin *et al.*, 1998
Garcinia cambogia			
Garbogiol **[54]**	Root	ND	Iinuma *et al.*, 1998
Rheediaxanthone A **[55]**	Bark	ND	Iinuma *et al.*, 1998
Garcinia cowa			
1,3,6–Trihydroxy–7–methoxy –2,5–bis (3–methyl–2–butenyl) xanthone **[56]**	Latex	antimicrobial	na Pattalung *et al.*, 1994
Norcowanin **[57]**	Latex	antimicrobial	na Pattalung *et al.*, 1994
7–*O*–methylgarcinone E b**[58]**	Bark	antimalarial (*P. falciparum*, IC_{50}=2.50 μg/ml)	Likhitwitayawuid, Phadungcharoen and Krungkrai, 1998 b
β–Mangostin **[37]**	Bark	antimalarial (*P. falciparum*, IC_{50}=3.00 μg/ml)	Likhitwitayawuid, Phadungcharoen and Krungkrai, 1998 b
Cowanin **[59]**	Latex Bark	antimicrobial antimalarial (*P. falciparum*, IC_{50}=3.00 μg/ml)	na Pattalung *et al.*, 1994; Likhitwitayawuid, Phadungcharoen and Krungkrai, 1998 b
Cowanol **[60]**	Latex Bark	antimicrobial antimalarial (*P. falciparum*, IC_{50}=1.60 μg/ml)	na Pattalung *et al.*, 1994; Likhitwitayawuid, Phadungcharoen and Krungkrai, 1998 b
Cowaxanthone **[61]**	Latex Bark	antimicrobial antimalarial (*P. falciparum*, IC_{50}=1.50 μg/ml)	na Pattalung *et al.*, 1994; Likhitwitayawuid, Phadungcharoen and Krungkrai, 1998 b

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Garcinia dulcis			
Dulciol A **[62]**	Bark	ND	Iinuma *et al*., 1996 b
Dulciol B **[63]**	Root	ND	Iinuma *et al*., 1996 b
Dulciol C **[64]**	Root	ND	Iinuma *et al*., 1996 b
Dulciol D **[65]**	Root	ND	Iinuma *et al*., 1996 b
Dulciol E **[66]**	Root	ND	Iinuma *et al*., 1996 b
1,7–Dihydroxyxanthone **[1]**	Bark	antimalarial (*P. falciparum*, IC_{50}=3.88 μg/ml)	Likhitwitayawuid *et al*., 1998 a
12b–Hydroxy–des–D–garcigerrin A **[67]**	Bark	antimalarial (*P. falciparum*, IC_{50}=2.08 μg/ml)	Likhitwitayawuid *et al*., 1998 a
1–*O*–Methylsymphoxanthone **[68]**	Bark	antimalarial (*P. falciparum*, IC_{50}=3.71 μg/ml)	Likhitwitayawuid *et al*., 1998 a
Symphoxanthone **[69]**	Bark	antimalarial (*P. falciparum*, IC_{50}=3.75 μg/ml)	Likhitwitayawuid *et al*., 1998 a
Garciniaxanthone **[70]**	Bark	antimalarial (*P. falciparum*, IC_{50}=0.96 μg/ml)	Likhitwitayawuid *et al*., 1998 a
Dulxanthone E **[71]**	leaf	ND	Kosela *et al*., 1999
Dulxanthone F **[72]**	leaf	ND	Kosela *et al*., 2000
Dulxanthone G **[73]**	leaf	ND	Kosela *et al*., 2000
Dulxanthone H **[74]**	leaf	ND	Kosela *et al*., 2000
Garcinia forbesii			
1,3,7–Trihydroxy–2–(3–methylbut–2–enyl)–anthoneb **[75]**	Twig and branch	ND	Harrison *et al*., 1993

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Garcinia forbesii			
Pyranojacareubin **[76]**	Twig and branch	ND	Harrison *et al*., 1993
Forbexanthone **[77]**	Twig and branch	ND	Harrison *et al*., 1993
Garcinia mangostana			
Mangostinone **[78]**	Fruit hull (pericarp)	ND	Asai *et al*., 1995
Mangostanol **[79]**	Fruit hull	cAMP phosphodiesterase inhibition	Chairungsrilerd *et al*., 1996
α–Mangostin **[80]**	Fruit hull	cAMP phosphodiesterase inhibition	Chairungsrilerd *et al*., 1996
γ–Mangostin **[81]**	Fruit hull	cAMP phosphodiesterase inhibition	Chairungsrilerd *et al*., 1996
Gartanin **[82]**	Fruit hull	ND	Chairungsrilerd *et al*., 1996
8–Deoxygartanin **[83]**	Fruit hull	ND	Chairungsrilerd *et al*., 1996
Garcinone E **[84]**	Fruit hull	ND	Chairungsrilerd *et al*., 1996
Garcimangosone A **[85**]	Fruit hull	ND	Huang *et al*., 2001
Garcimangosone B **[86]**	Fruit hull	ND	Huang *et al*., 2001
Garcimangosone C **[87]**	Fruit hull	ND	Huang *et al*., 2001
Garcinia parvifolia			
Parvixanthone A **[88]**	Bark	ND	Xu *et al*., 2001
Parvixanthone B **[89]**	Bark	ND	Xu *et al*., 2001

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Garcinia parvifolia			
Parvixanthone C **[90]**	Bark	ND	Xu *et al*., 2001
Parvixanthone D **[91]**	Bark	ND	Xu *et al*., 2001
Parvixanthone E**[92]**	Bark	ND	Xu *et al*., 2001
Parvixanthone F **[93]**	Bark	ND	Xu *et al*., 2001
Parvixanthone G **[94]**	Bark	ND	Xu *et al*., 2001
Parvixanthone H **[95]**	Bark	ND	Xu *et al*., 2001
Parvixanthone I **[96]**	Bark	ND	Xu *et al*., 2001
Garcinia vilersiana			
Globuxanthone **[97]**	Bark	ND	Nguyen and Harrison, 2000
Subelliptenone H **[98]**	Bark	ND	Nguyen and Harrison, 2000
Subelliptenone B **[99]**	Bark	ND	Nguyen and Harrison, 2000
12b–Hydroxy–des–D–garcigerrin A **[67]**	Bark	ND	Nguyen and Harrison, 2000
Symphoxanthone **[69]**	Bark	ND	Nguyen and Harrison, 2000
1–*O*–Methylglobuxanthone **[100]**	Bark	ND	Nguyen and Harrison, 2000
Hypericum ascyron			
2–Methoxyxanthone **[101]**	Aerial part	ND	Hu, Yip, and Sim, 1999
1,7–Dihydroxyxanthone **[1]**	Aerial part	ND	Hu, Yip, and Sim, 1999
1–Hydroxy–7–methoxy xanthone **[102]**	Aerial part	ND	Hu, Yip, and Sim, 1999
3,6–Dihydroxy–1,7–dimethoxyxanthone **[103]**	Aerial part	ND	Hu, Yip, and Sim, 1999

Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Hypericum ascyron			
7–Methoxy–1,5,6–trihydroxy xanthone [104]	Aerial part	ND	Hu, Yip, and Sim, 1999
Hypericum canariensis			
Hydroxanthone [34]	Aerial part	ND	Cardona *et al*., 1986
1,7–Dihydroxyxanthone [1]	Aerial part	ND	Cardona *et al*., 1986
2–Hydroxy–5–methoxy xanthone **[105]**	Aerial part	ND	Cardona *et al*., 1986
2,5–Dihydroxyxanthone **[106]**	Aerial part	ND	Cardona *et al*., 1986
Hypericum ericoides			
2,3,4–Trihydroxyxanthone **[107]**	Stem, leaf and flower	ND	Cardona and Seoane, 1982
2–Hydroxyxanthone **[34]**	Stem, leaf and flower	ND	Cardona and Seoane, 1982
2–Hydroxy–5,6,7–trimethoxy xanthone **[108]**	Stem, leaf and flower	ND	Cardona and Seoane, 1982
Hypericum reflexum			
*Hyperireflexin **[109]***	Aerial part	ND	Cardona *et al*., 1990
Hyperxanthone **[110]**	Aerial part	ND	Cardona *et al*., 1990
4–Hydroxy–2,3–dimethoxy xanthone **[111]**	Aerial part	ND	Cardona *et al*., 1990
3,4–Dihydroxy–2–methoxy xanthone **[112]**	Aerial part	ND	Cardona *et al*., 1990
3–Hydroxy–2,4–dimethoxy xanthone **[113]**	Aerial part	ND	Cardona *et al*., 1990
4–Hydroxy–2,3,6–trimethoxy xanthone **[114]**	Aerial part	ND	Cardona *et al*., 1990

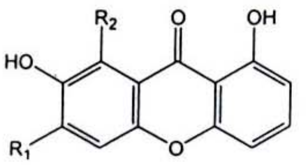
Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Hypericum patulum			
*Paxanthone B **[115]***	Callus tissue	ND	Ishiguro *et al.*, 1995
*Garcinone B **[116]***	Callus tissue	ND	Ishiguro *et al.*, 1995
1,3,6,7–Tetrahydroxy–8–(3–methyl–2–butenyl) –9H–xanthene–9–one **[117]**	Callus tissue	ND	Ishiguro *et al.*, 1995
Mammea acuminata			
2,7–Dihydroxyxanthone **[118]**	Stem and bark	ND	Tosa *et al.*, 1997
1,5–Dihydroxyxanthone **[13]**	Stem and bark	ND	Tosa *et al.*, 1997
5–Hydroxy–1–methoxy xanthone [**119]**	Stem and bark	ND	Tosa *et al.*, 1997
2,4–Dihydroxy–3,6–dimethoxyxanthone **[120]**	Aerial part	ND	Cardona *et al.*, 1990
3,6–Dihydroxy–2–methoxy xanthone **[121]**	Aerial part	ND	Cardona *et al.*, 1990
Mammea siamensis			
5–Hydroxy–1–methoxy xanthone **[119]**	Twig	ND	Poobrasert *et al.*, 1998
1,3–Dimethoxy–5–hydroxy xanthone **[122]**	Twig	ND	Poobrasert *et al.*, 1998
1,2–Dimethoxy–5–hydroxy xanthone **[123]**	Twig	ND	Poobrasert *et al.*, 1998
1,7–Dihydroxyxanthone **[1]**	Twig	ND	Poobrasert *et al.*, 1998
2,5–Dihydroxy–1–methoxy xanthone [**124]**	Twig	ND	Poobrasert *et al.*, 1998
1,3,7–Trihydroxyxanthone **[15]**	Twig	ND	Poobrasert *et al.*, 1998

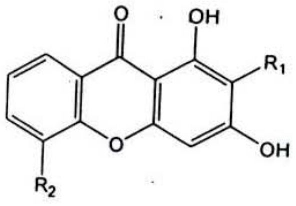
Table 1 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Mammea siamensis			
3,5–Dihydroxy–1–methoxy xanthone **[125]**	Twig	ND	Poobrasert *et al.*, 1998
Mesua ferrea			
1,3–Dimethoxy–5,6–dihydroxyxanthone (ferrxanthone) **[126]**	Heartwood	ND	Walia and Mukerjee, 1984
Mesuferrol A [**127]**	Bark	ND	Iinuma *et al*, 1996 c
Mesuferrol B **[128]**	Bark	ND	Iinuma *et al*, 1996 c
1,7–Dihydroxyxanthone **[1]**	Bark	ND	Iinuma *et al*, 1996 c
5–Hydroxy–1–methoxy xanthone **[119]**	Bark	ND	Iinuma *et al*, 1996 c
Ploiarium alternifolium			
Ploiarixanthone ***[129]***	Branch	ND	Bennett *et al.*, 1990
	Bark	ND	Bennett *et al.*, 1991

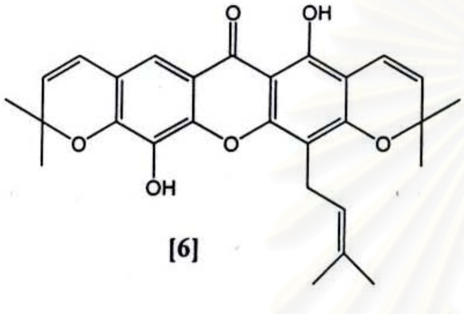
ND no data



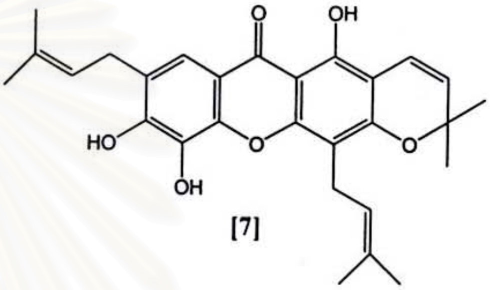
	R$_1$	R$_2$
[1]	H	H
[2]	H	OH
[3]	OH	OH



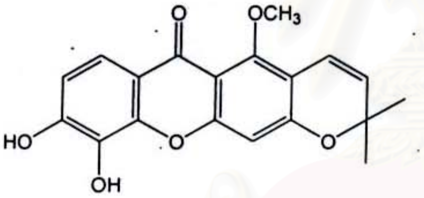
	R$_1$	R$_2$
[4]	OH	OH
[5]	OCH$_3$	OCH$_3$



[6]



[7]



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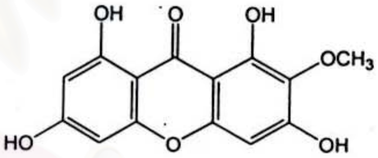

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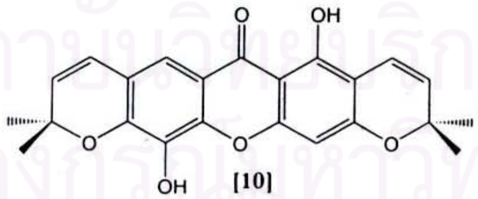
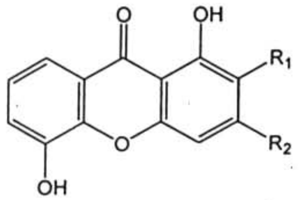
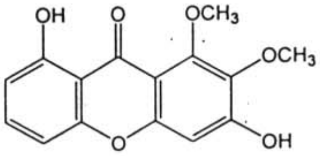


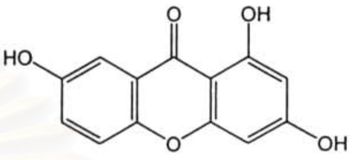
[10]



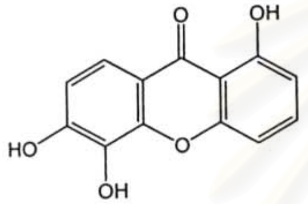
	R_1	R_2
[11]	OCH_3	OH
[12]	H	OH
[13]	H	H



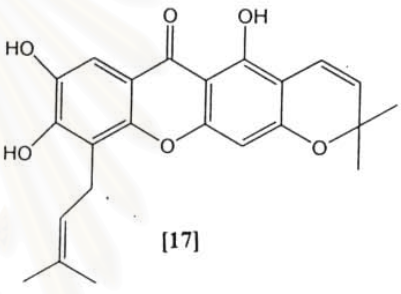
[14]



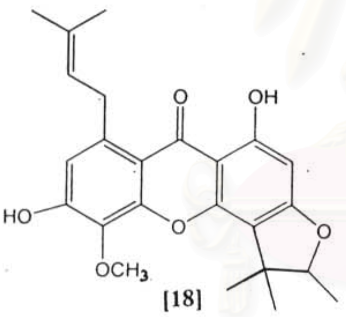
[15]



[16]



[17]



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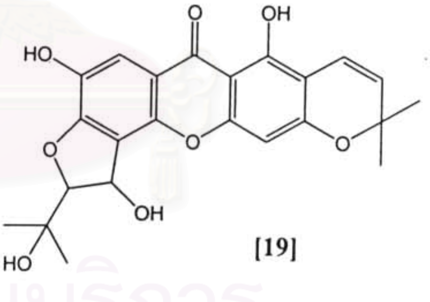

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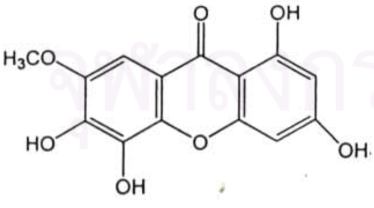

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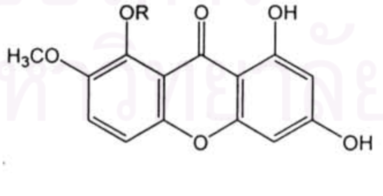
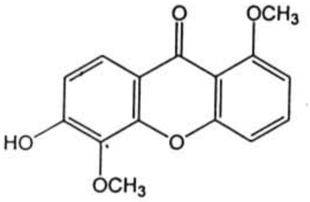
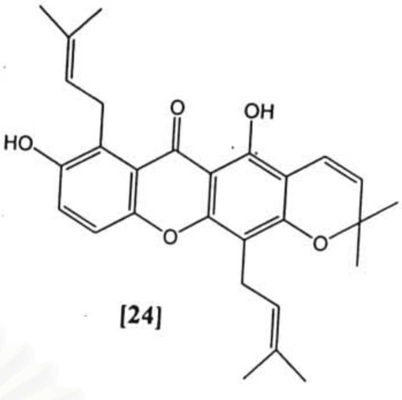


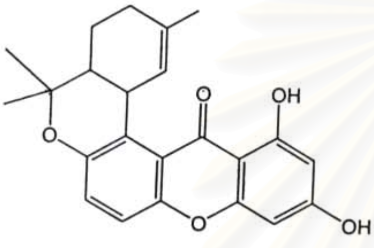
	R
[21]	H
[22]	CH_3



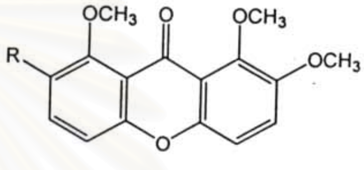
[23]



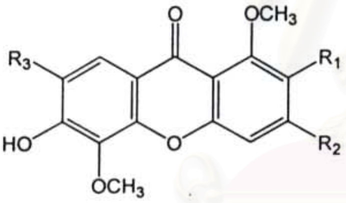
[24]



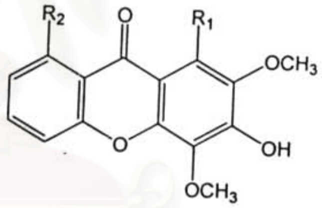
[25]



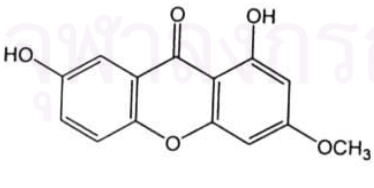
	R
[26]	H
[28]	OH



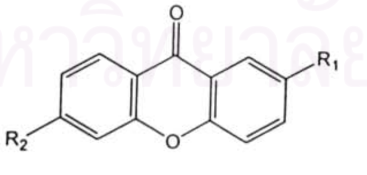
	R_1	R_2	R_3
[27]	H	OCH_3	OCH_3
[29]	OCH_3	H	H



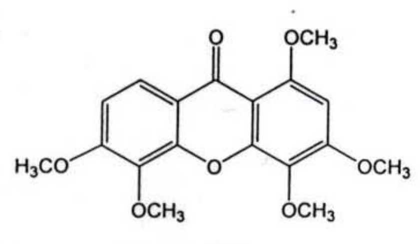
	R_1	R_2
[30]	OCH_3	OH
[31]	H	H



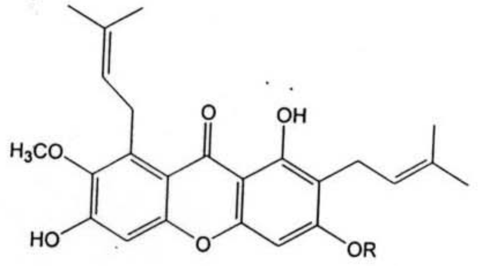
[32]



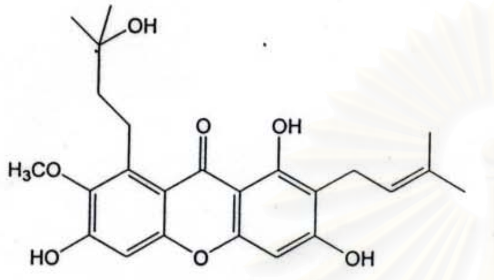
	R_1	R_2
[33]	$COOCH_3$	OCH_3
[34]	OH	H



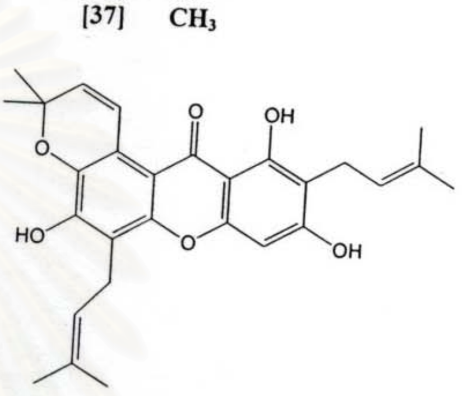
[35]



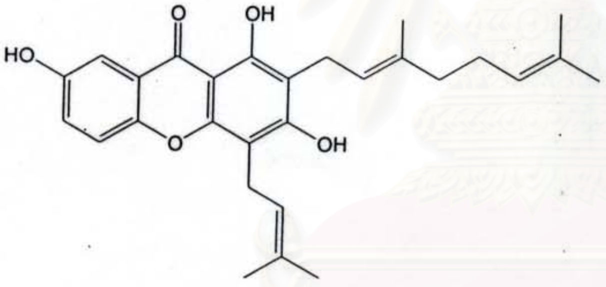
	R
[36]	H
[37]	CH_3



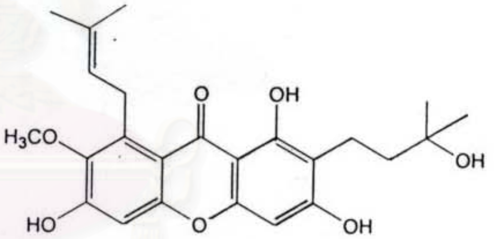
[38]



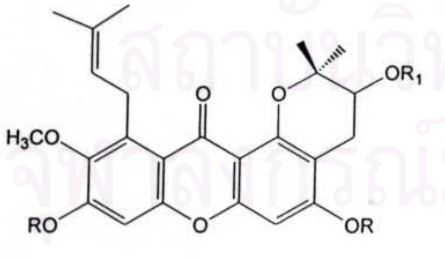
[39]



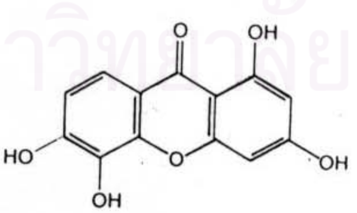
[40]



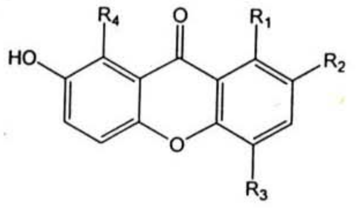
[41]



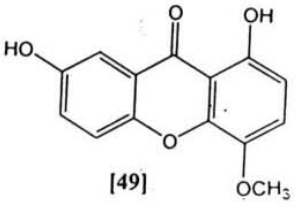
	R	R_1
[42]	H	H
[43]	CH_3	H
[44]	CH_3	Ac



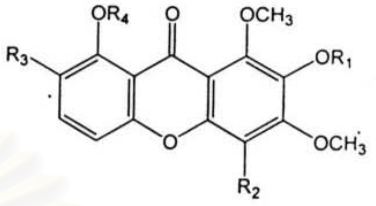
[45]



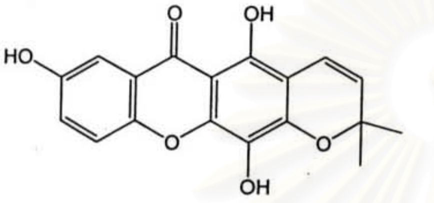
	R1	R2	R3	R4
[46]	OCH_3	OH	H	OCH_3
[47]	OH	H	OH	OCH_3
[48]	OH	H	OH	H



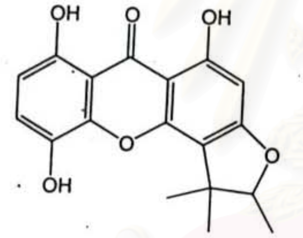
[49]



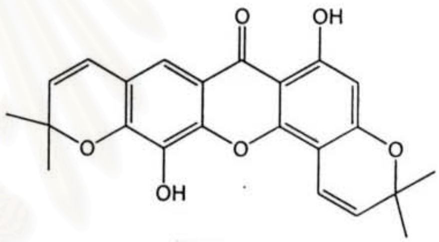
	R1	R2	R3	R4
[50]	CH_3	OCH_3	H	CH_3
[51]	H	H	H	H
[52]	CH_3	H	OH	CH_3



[53]



[54]



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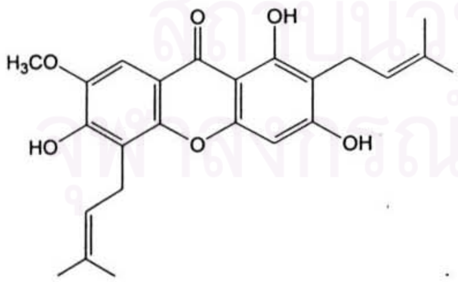

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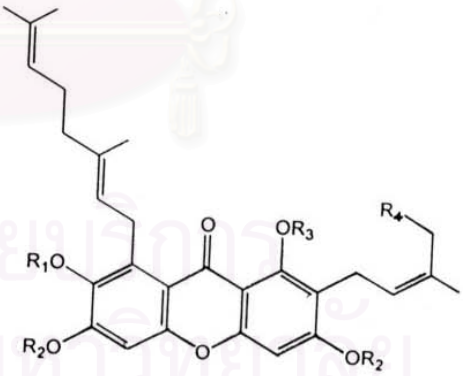
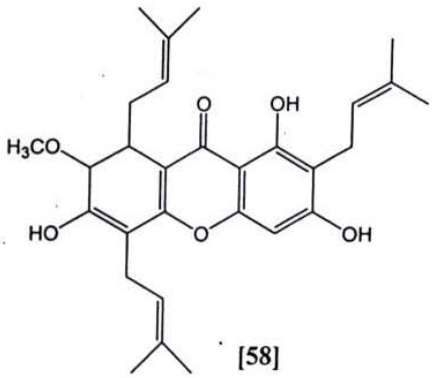
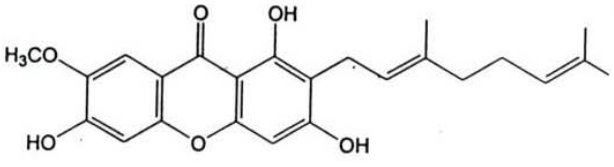


	R1	R2	R3	R4
[57]	H	H	H	H
[59]	CH_3	H	H	H
[60]	CH_3	H	H	OH



[58]



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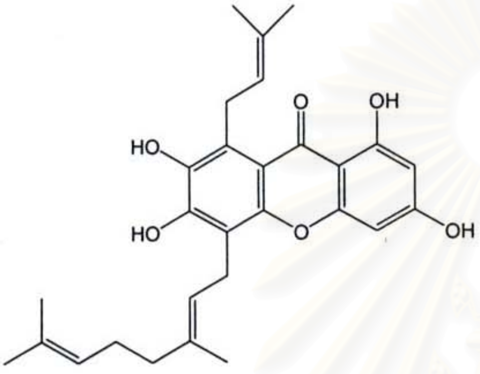

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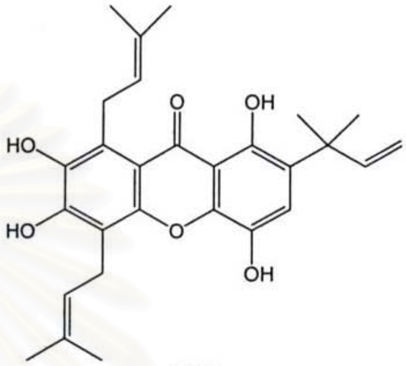

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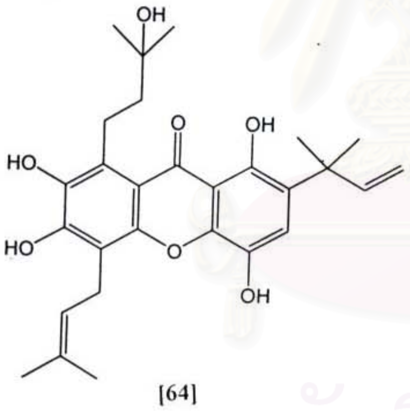

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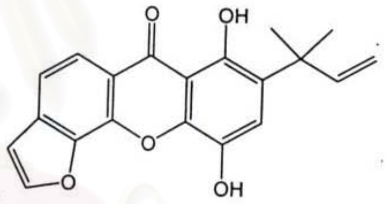

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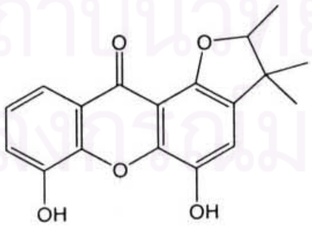

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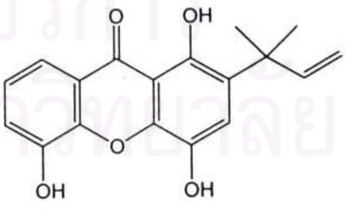
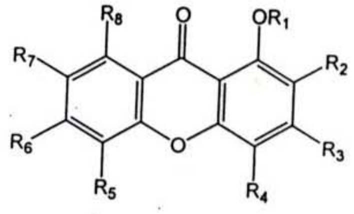
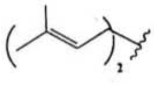
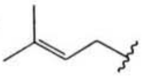
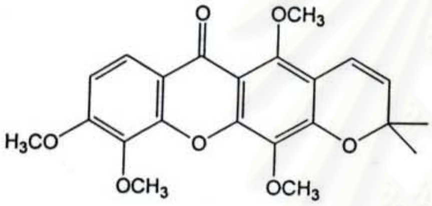


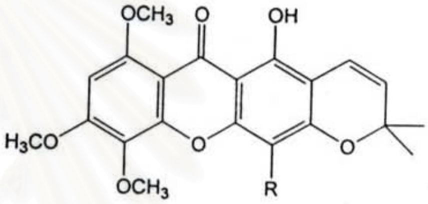
[67]



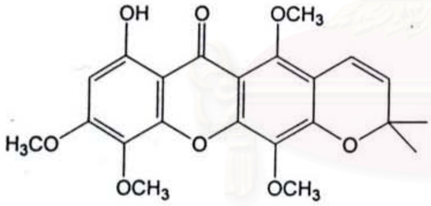
	R1	R2	R3	R4	R5	R6	R7	R8
[68]	CH_3	OH	H		OH	OH	H	H
[69]	H	OH	H		OH	OH	H	H
[70]	H	H	OH	H	OH	OH		



[71]



	R
[72]	H
[73]	OCH_3



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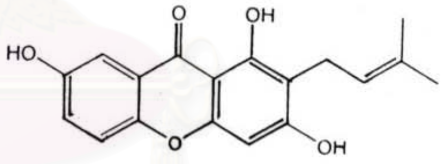

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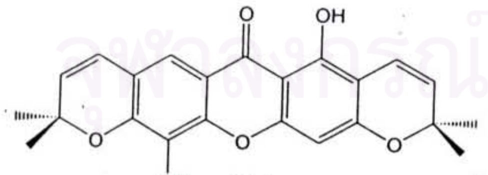

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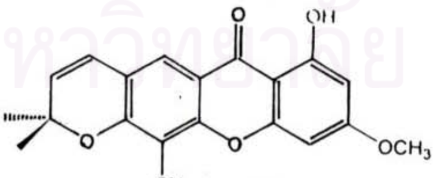

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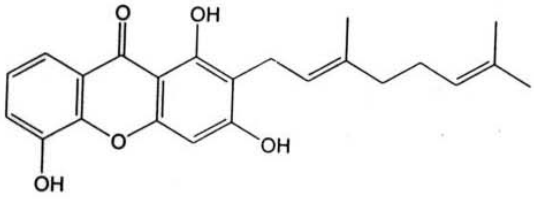

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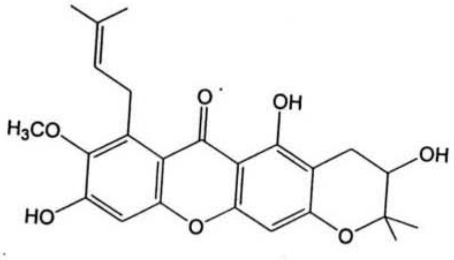
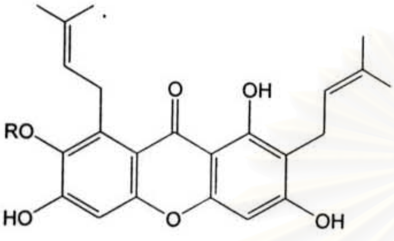
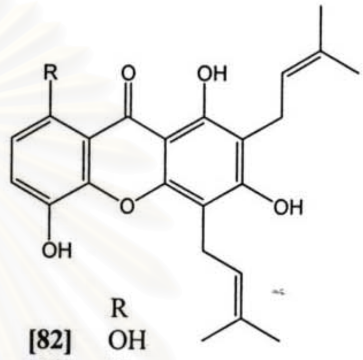


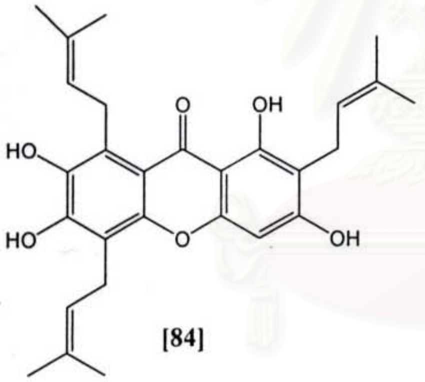
[79]



	R
[80]	CH_3
[81]	H



	R
[82]	OH
[83]	H



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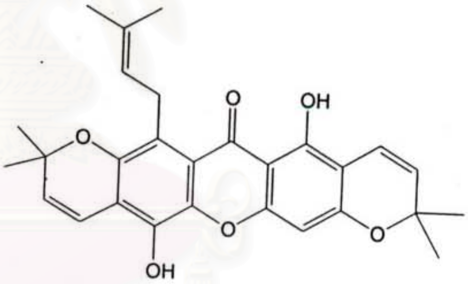

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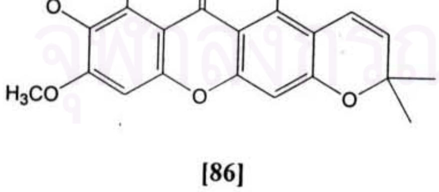

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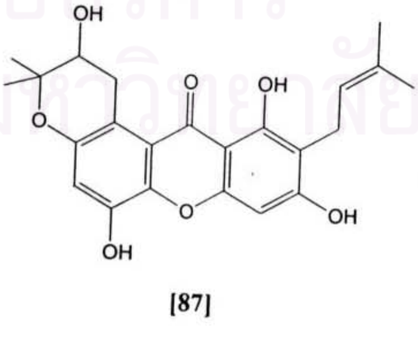

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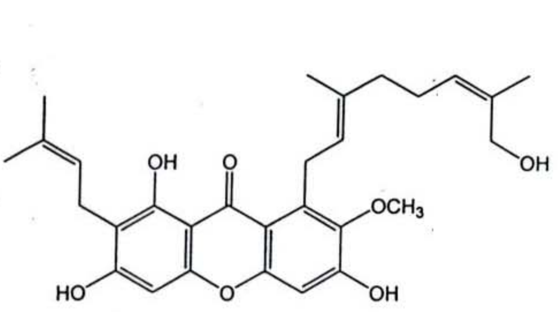

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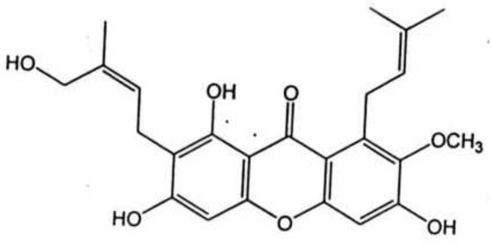

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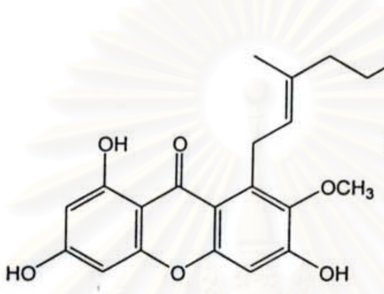

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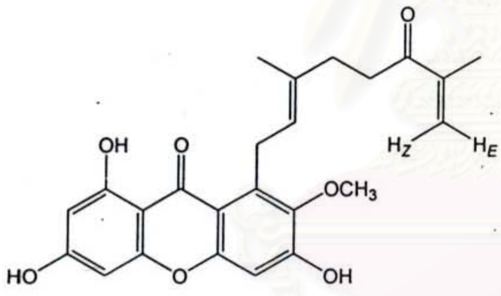

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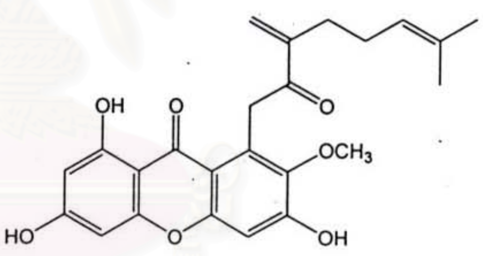

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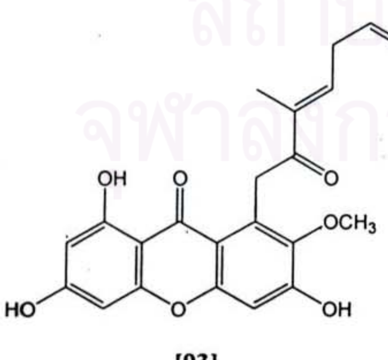

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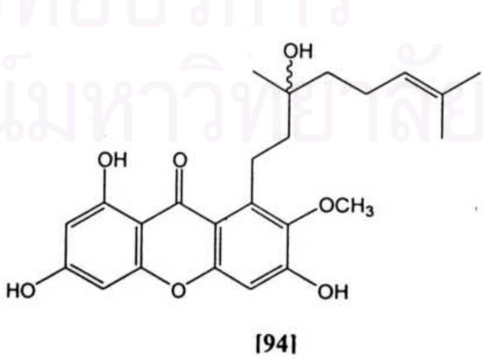

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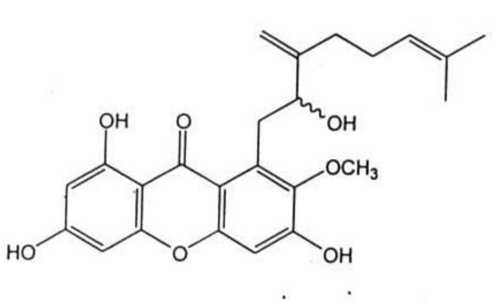

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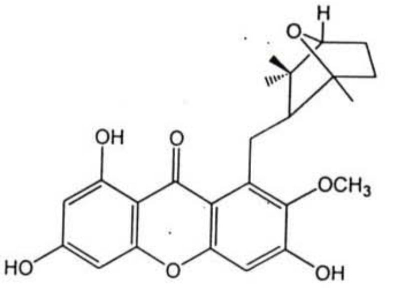

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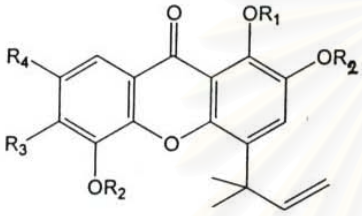
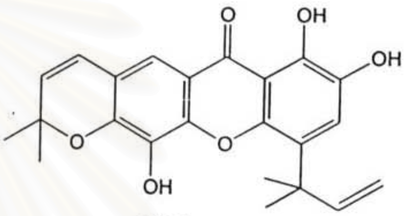
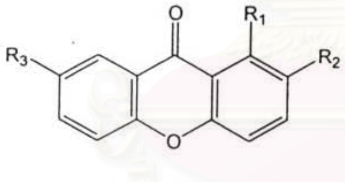


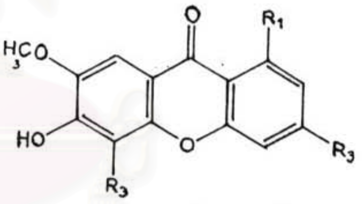


[98]

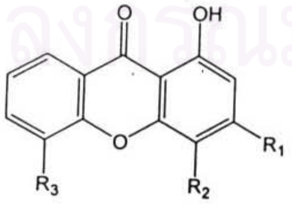
	R_1	R_2	R_3	R_4
[97]	H	H	H	H
[99]	H	H	H	3-methylbut-2-enyl
[100]	CH_3	H	H	H



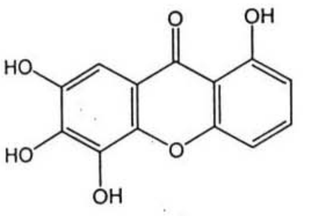
	R_1	R_2	R_3
[101]	H	OCH_3	H
[102]	OH	H	OCH_3



	R_1	R_2	R_3
[103]	OCH_3	OH	H
[104]	OH	H	OH



	R_1	R_2	R_3
[105]	H	H	OCH_3
[106]	H	H	OH
[107]	OH	OH	H



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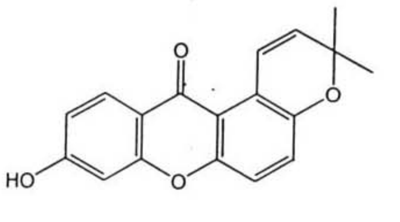

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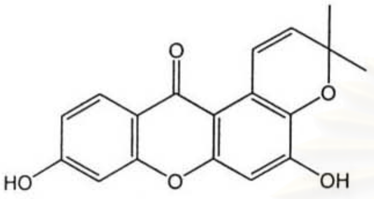

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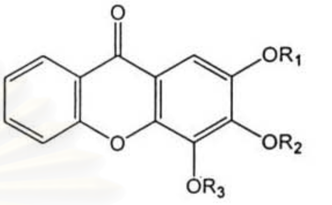
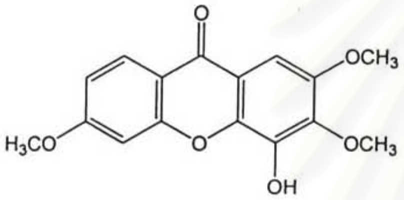


	R_1	R_2	R_3
[111]	CH_3	CH_3	H
[112]	CH_3	H	H
[113]	CH_3	H	CH_3



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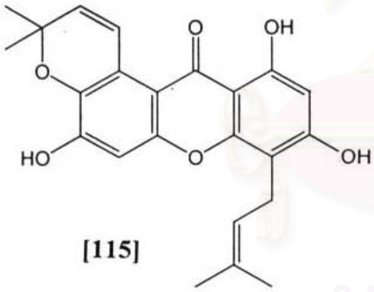

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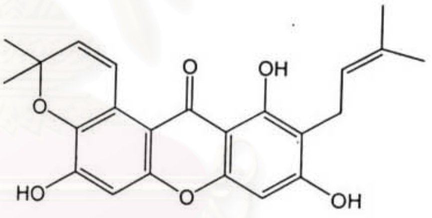
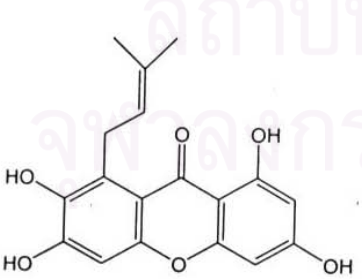


[116]



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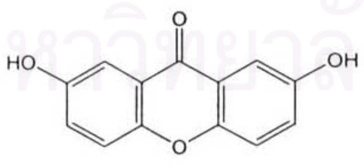

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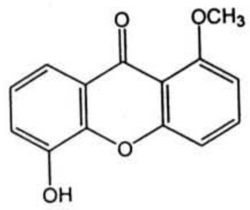

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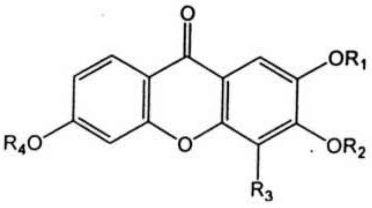
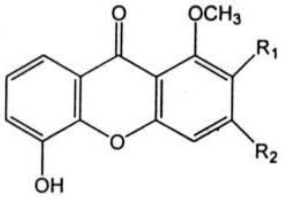
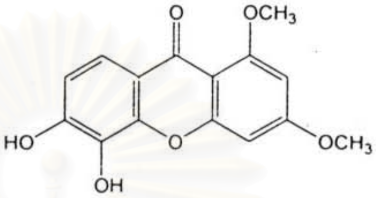


	R₁	R₂	R₃	R₄
[120]	**H**	**CH_3**	**OH**	**CH_3**
[121]	**CH_3**	**H**	**H**	**H**



	R₁	R₂
[122]	**H**	**OCH_3**
[123]	**OCH_3**	**H**
[124]	**OH**	**H**
[125]	**H**	**OH**



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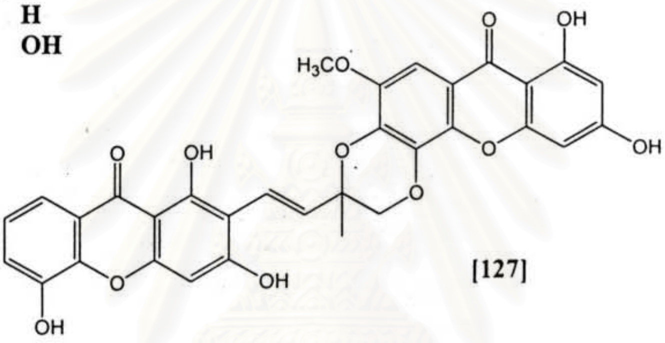

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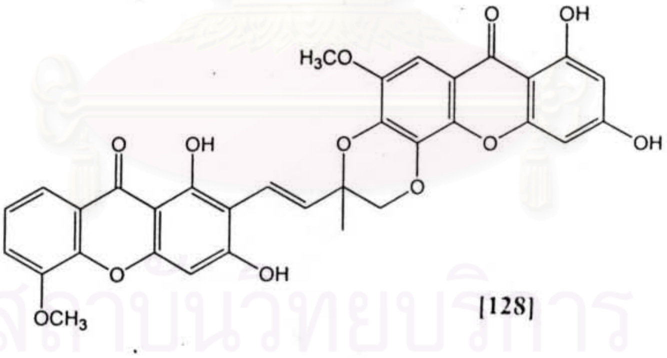

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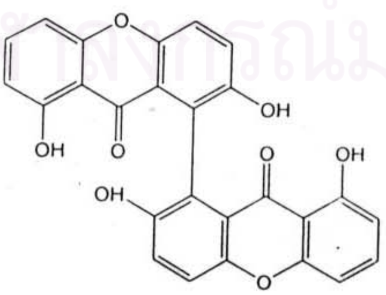


[129]

Table 2 Distribution of flavonoids in Guttiferae.

Plant and chemical compound	Plant part	Activity	Reference
Clusia nemorosa			
Kaempferol **[130]**	Bark	Anti–HIV–1 RT	Lin *et al*., 1997 ; De Andrade, Almeida and Conserva, 1998
Cratoxylum formosanum			
(–)–Epicatechin **[131]**	Root	ND	Iinuma *et al*., 1996 a
Astilbin **[132]**	Root	ND	Iinuma *et al*., 1996 a
Garcinia andamanica			
Sorbifolin 6–galactoside**[133]**	Leaf	ND	Alam *et al*.,1986
Scutellarein 7–diglucoside **[134]**	Leaf	ND	Alam *et al*.,1986
Garcinia kola			
GB–1 **[135]**	Seed Stem bark	ND ND	Kabangu *et al*., 1987 Kabangu *et al*., 1987
GB–2 **[136]**	Seed Stem bark	ND ND	Kabangu *et al*., 1987 Kabangu *et al*., 1987
Kolaflavanone **[137]**	Seed	ND	Kabangu *et al*., 1987
GB–3 **[138]**	Stem bark	ND	Kabangu *et al*., 1987
GB–1a **[139]**	Seed	Anti–HIV–1 RT (IC_{50}236 μM)	Kabangu *et al*., 1987; Lin *et al*., 1997
Garcinia multiflora			
GB–1a **[139]**	Heartwood	Anti–HIV–1 RT(IC_{50} 236 μM)	Lin *et al*., 1997
GB–1a–7″–*O*–glucoside **[140]**	Heartwood	No activity	Lin *et al*., 1997
GB–2a **[141]**	Heartwood	Anti–HIV–1 RT(IC_{50} 170 μM)	Lin *et al*., 1997

Table 2 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Garcinia multiflora			
Volkensiflavone **[142]**	Heartwood	Anti–HIV–1 RT(weakly active)	Lin *et al.*, 1997
Morelloflavone **[143]**	Heartwood	Anti–HIV–1 RT(IC_{50} 116 μM)	Lin *et al.*, 1997
Xanthochymuside **[1444]**	Heartwood	ND	Lin *et al.*, 1997
Spicataside **[145]**	Heartwood	ND	Lin *et al.*, 1997
Fukagiside **[146]**	Heartwood	ND	Lin *et al.*, 1997
Hypericum brasiliense			
Kaempferol **[130]**	Flower and leaf	Anti–HIV–1 RT	Rocha *et al.*, 1995; Lin *et al.*, 1997
Quercetin **[147]**	Flower and leaf	Anti–HIV–1 RT	Rocha *et al.*, 1995; Lin *et al.*, 1997
Quercitrin **[148]**	Flower and leaf	ND	Rocha *et al.*, 1995
Isoquercitrin **[149]**	Flower and leaf	ND	Rocha *et al.*, 1995
Hypericum elodes			
Quercetin 3–glucuronide–3′–sulphate **[150]**	Aerial part	ND	Sebra and Alves, 1988
*Quercetin 3′–sulphate **[151]***	Aerial part	ND	Sebra and Alves, 1988
Hypericum japonicum			
*Taxifolin 7–rhamnoside **[152]***	Whole plant	ND	Ishiguro *et al.*, 1991
Quercitrin **[148]**	Whole plant	ND	Ishiguro *et al.*, 1991
Vincetoxicoside B **[153]**	Whole plant	ND	Ishiguro *et al.*, 1991
Salothranol **[154]**	Whole plant	Antibacterial (*S. aureus*, MIC 125 μg/ml)	Ishiguro *et al.*, 1993

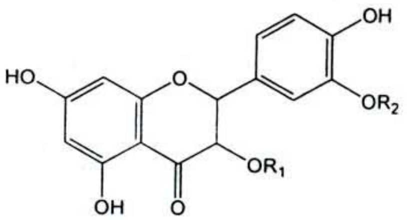
Table 2 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Hypericum japonicum			
7,8– (2″,2″–Dimethylpyrano) 5,3′,4′–trihydroxy–3–methoxyflavone **[155]**	Aerial part	ND	Wu *et al.*, 1998
(2R,3R) Dihydroquercetin–3, 7–*O*–*α*–L–dirhamnoside **[156]**	Aerial part	ND	Wu *et al.*, 1998
(2R,3R) Dihydroquercetin–7–*O*–*α*–L–rhamnoside **[157]**	Aerial part	Coagulation of APTT	Wu *et al.*, 1998
(2R,3R) Dihydroquercetin **[158]**	Aerial part	ND	Wu *et al.*, 1998
2,3–*trans*–Dihydro–3,5,4′–trihydroxyflavonol 7–*O*–*α*–L–rhamnoside **[159]**	Aerial part	ND	Wu *et al.*, 1998
Quercetin–3–*O*–*β*–D–glucoside **[160]**	Aerial part	Anticoagulation of APTT	Wu *et al.*, 1998
Quercetin–7–*O*–*α*–L–rhamnoside **[161]**	Aerial part	Promoting coagulation of APTT, PT	Wu *et al.*, 1998
Quercetin–3–*O*–*α*–L–rhamnosyl (1→2) –*O*–*α*–L–rhamnoside **[162]**	Aerial part	Anticoagulation of APTT	Wu *et al.*, 1998
*Quercetin **[1147]***	Aerial part	Promoting coagulation of APTT, PT Anti–HIV–1 RT	Lin *et al.*, 1997; Wu *et al.*, 1998
Kaempferol **[130]**	Aerial part	Anti–HIV–1 RT	Lin *et al.*, 1997; Wu *et al.*, 1998
Kaempferol–7–*O*–*α*–L–rhamnoside **[163]**	Aerial part	ND	Wu *et al.*, 1998
Hypericum perforatum			
*Hyperoside [**164]***	Callus	ND	Dias *et al.*, 1998

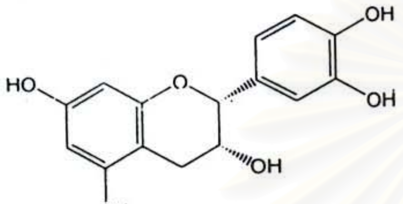
Table 2 (continued)

Plant and chemical compound	Plant part	Activity	Reference
Hypericum perforatum			
I3, II8–Biapigenin ***[165]***	Whole plant	ND	Borghöfer and Hoelzl,1987
Amentoflavone ***[166]***	Whole plant	ND	Borghöfer and Hoelzl,1989
Luteolin 5 – Glucoside ***[167]***	Callus	ND	Dias *et al*., 1998
Luteolin 3′ – Glucoside ***[168]***	Callus	ND	Dias *et al*., 1998
*Luteolin [****169]***	Callus	ND	Dias *et al*., 1998

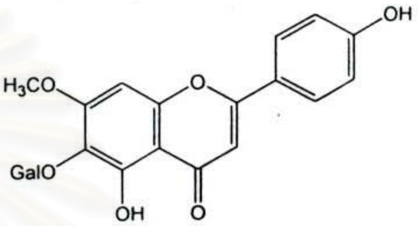
ND	no data
APTT	Activated Partial Thromboplastin Time Reagent
PT	Prothrombin Time Reagent
HIV-1 RT	Human immunodeficiency virus-1 reverse transcriptase



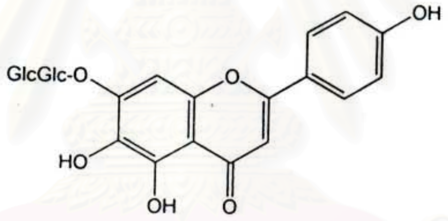
	R_1	R_2
[130]	H	H
[132]	Rha	OH



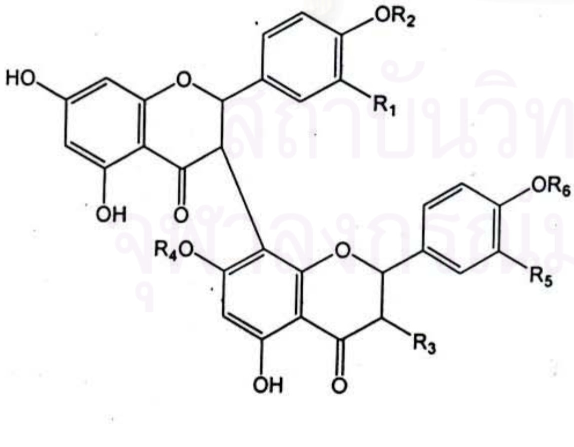
[131]



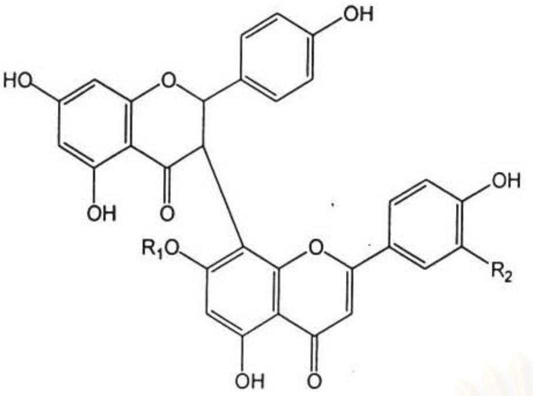
[133]



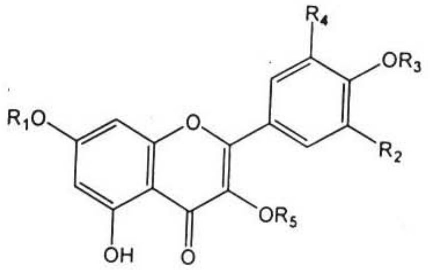
[134]



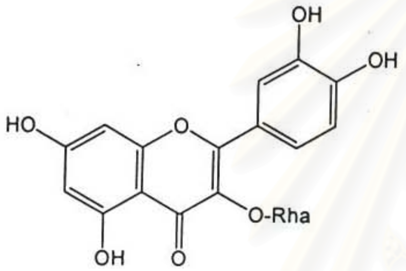
	R_1	R_2	R_3	R_4	R_5	R_6
[135]	H	H	OH	H	H	H
[136]	H	H	OH	H	OH	H
[137]	H	H	OH	H	OH	CH_3
[138]	OH	CH_3	OH	H	OH	H
[139]	H	H	H	H	H	H
[140]	H	H	H	Glc	H	H
[141]	H	H	H	H	OH	H
[144]	H	H	OH	Glc	OH	H



	R₁	R₂
[142]	H	H
[143]	H	OH
[145]	Glc	H
[146]	Glc	OH



	R₁	R₂	R₃	R₄	R₅
[147]	H	H	H	OH	H
[150]	H	H	H	OSO_3K	Glucoronide
[151]	H	H	H	OSO_3K	H
[153]	Rha	CH_3	H	OH	H



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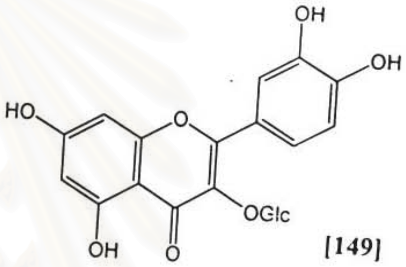
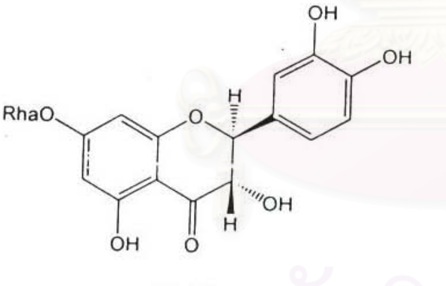
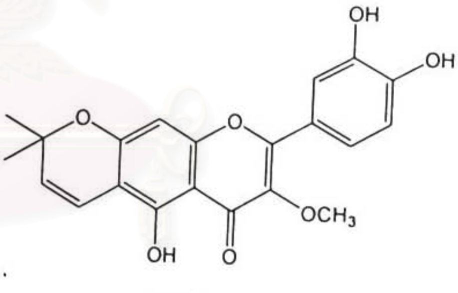


[149]



[152]



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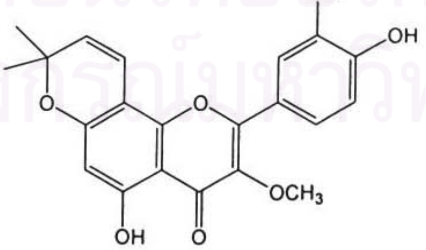
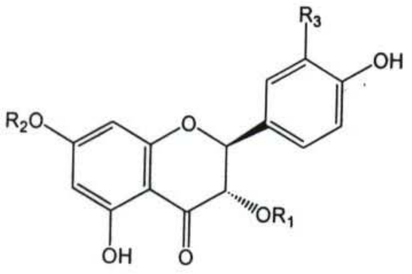
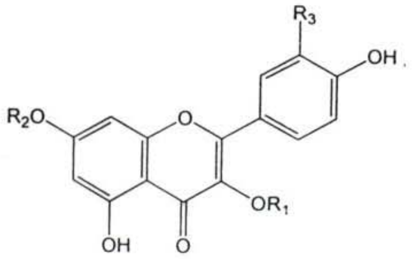


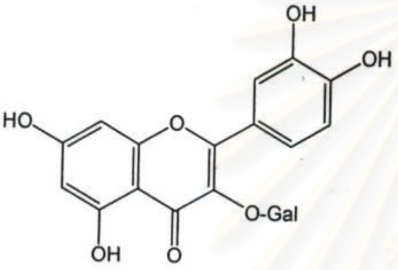
[155]



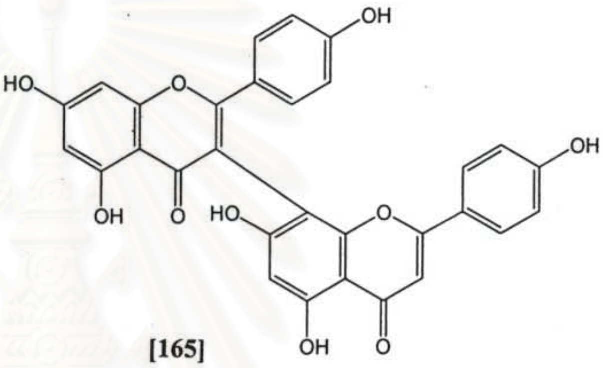
	R1	R2	R3
[156]	**Rha**	**Rha**	**OH**
[157]	**H**	**Rha**	**OH**
[158]	**H**	**H**	**OH**
[159]	**H**	**Rha**	**H**



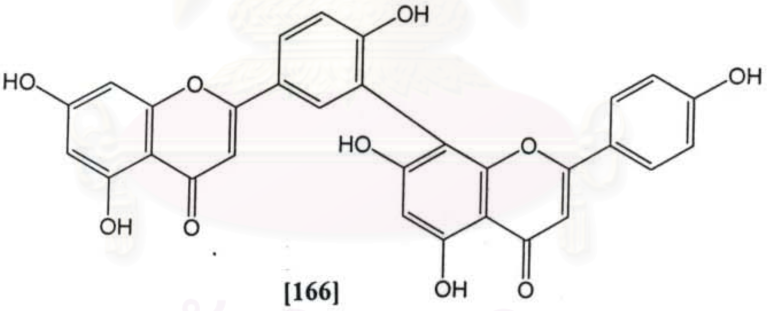
	R1	R2	R3
[160]	Glc	H	OH
[161]	H	Rha	OH
[162]	Rha (2→ 1) Rha	H	OH
[163]	H	Rha	H



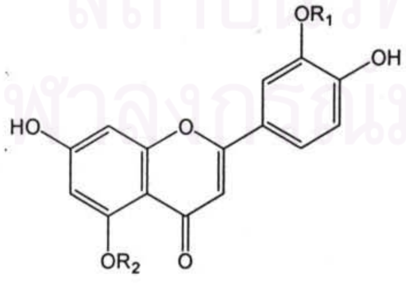
[164]



[165]



[166]

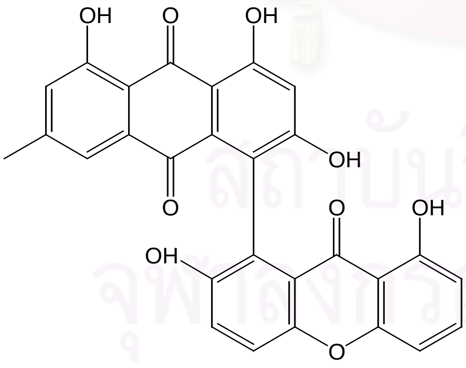


	R1	R2
[167]	H	Glu
[168]	Glu	H
[169]	H	H

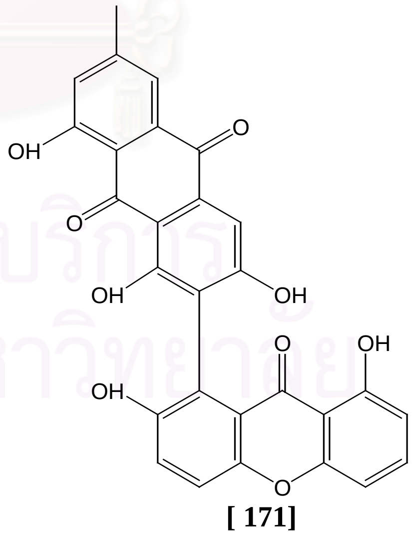
3. Chemical constituents of the genera *Ploiarium* and *Bonnetia*

Phytochemical studies of *Ploiarium alternifolium* were previously reported during the years 1990 to 1992. Isolation and purification of its branches showed the presence of one bixanthone, ploiarixanthone **[129]** and two anthraquinonyl xanthones namely euxanmodin A **[170]** and euxanmodin B **[171]** (Bennett *et al*., 1990). Further phytochemical studies reported that its bark also contained these compounds in addition to the emodin derivatives, ploiariquinone A **[172]** and ploiariquinone B **[173]** (Bennett *et al*., 1991). Moreover, two triterpenoid benzoates, methyl 3*β*-benzoyloxyoleana-11, 13(18)-dien-28-oate **[174]** and 3*β*-benzoyloxyolean-11-en-13*β*, 28-olide **[175]** were also found in the bark (Bennett *et al*., 1992). However, chemical constituent of other *Ploiarium* species has never been reported.

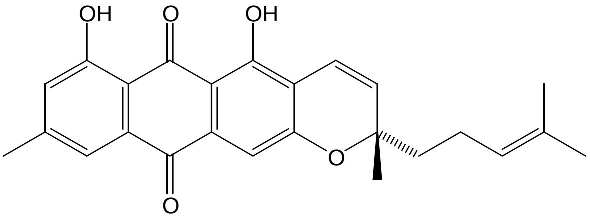
Another investigation was the phytochemical study of *Bonnetia dinizii* (Oliveira *et al*., 1990). Its wood was found to contain 1,7–dihydroxyxanthone **[1]**, 1,7,8–trihydroxyxanthone **[2]**, 1,6,7,8–tetrahydroxyxanthone **[3]**, 1,2,3,5 –tetrahydroxy xanthone **[4]**, and 1,3–dihydroxy–2,5 –dimethoxyxanthone **[5]**.



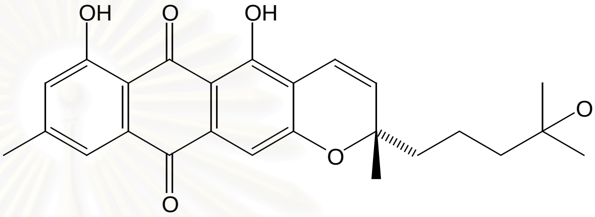
[170]



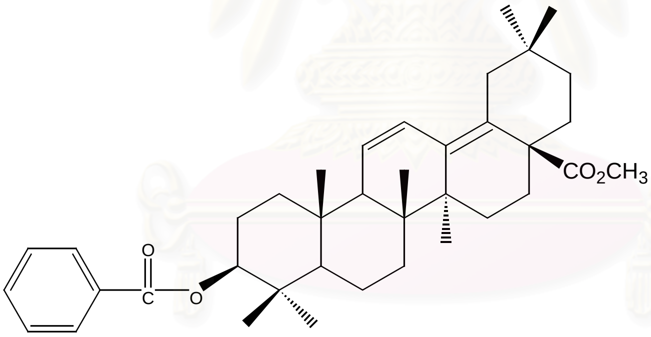
[171]



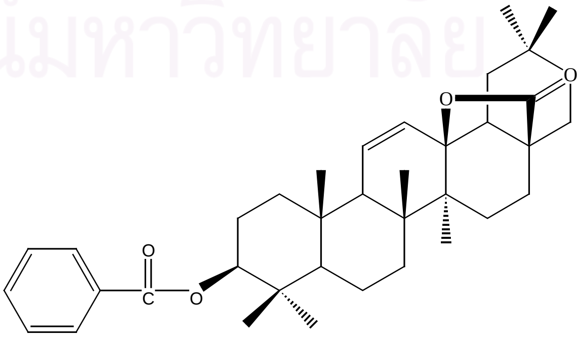
[172]



[173]



[174]



[175]

CHAPTER III

EXPERIMENTAL

1. Sources of plant material

The leaves of *Ploiarium alternifolium* (Vahl) Melchior were collected from the Peninsular Botanic Garden (Thung khai), Trang province, Thailand, in April 2000. Authentication of the plant material was done by comparison with herbarium specimens at the Royal Forest Department, Bangkok, Thailand.

2. General techniques

2.1 Analytical thin-layer chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	250 μm
Distance	:	5 cm
Temperature	:	Laboratory temperature (25-30 °C)
Detection	:	1. Visual detection under daylight 2. Ultraviolet light at wavelengths of 254 and 365 nm

2.2 Column chromatography

2.2.1 Quick column chromatography

Adsorbent	:	Silica gel 60 (No. 7734) particle size 0.063 - 0.200 mm(70-230 mesh ASTM) (E. Merck)
Packing method	:	Dry packing in the sintered glass filter column

Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.

Detection : Fractions were examined by TLC technique in the same manner as described in Section 2.1

2.2.2 Column chromatography

Adsorbent : Silica gel 60 (No. 7734) particle size 0.063-0.200 mm (70-230 mesh ASTM) (E. Merck)

Packing method : The adsorbent was slurried in the eluent, poured into a column and then allowed to settle overnight.

Sample loading : The sample was prepared as described above

Detection : Fractions were examined by TLC technique in the same manner as described in Section 2.1

2.3 High speed countercurrent chromatography

Instrument : High Speed Countercurrent Chromatograph P.C. Inc. model MKII , equipped with a polytetrafluoroethylene(PTFE) multilayer coil with a total capacity of 320 ml.

Solvent system : The two-phase solvent systems were composed of chloroform-methanol-water at various ratios. After thoroughly equilibrated the mixture, the two phases were separated shortly before use. The aqueous phase was used as the stationary phase and the organic phase as the mobile phase.

Sample loading : The sample was dissolved in equal volume of upper phase and lower phase and filtered before injection.

Detection : Fractions were examined by TLC technique in the same manner as described in Section 2.1

2.4 Crystallization technique

All compounds were crystallized from methanol. Each compound was dissolved in methanol until saturated and left standing at room temperature until yellow amorphous powder or yellow-needle crystals were formed.

2.5 Spectroscopy

2.5.1 Ultraviolet (UV) absorption spectra

Ultraviolet spectra were obtained from a Milton Roy Spectronic 3000 Array spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Infrared (IR) absorption spectra

IR spectra (KBr disc) were obtained on a Perkin-Elmer FTIR spectrometer GX (Division of Drugs Analysis, Department of Medical Sciences).

2.5.3 Mass spectra

Mass spectra were obtained by an Electrospray Ionization Time of Flight mass spectra (ESI-TOF MS) made on a Micromass LCT mass spectrometer. (The National Center for Genetic Engineering and Biotechnology, BIOTEC) and Fast Atom Bombardment mass spectrum (FAB-MS) on a JMS-DX302 mass spectrometer (Meiji Pharmaceutical University, Japan).

2.5.4 Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR spectra

^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), DEPT 135, COSY, HMQC, and HMBC spectra were recorded on a Bruker AVANCE DPX-300 FT-NMR spectrometer. Proton detected heteronuclear correlations were measured using HMQC (optimized for $^{n}J_{HC}$ = 145 Hz) and HMBC (optimized for $^{n}J_{HC}$ = 4 and 8 Hz) pulse sequences (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

The solvents for NMR spectra was deuterated dimethylsulfoxide (DMSO-d_6). The chemical shifts were reported in ppm scale using the chemical shift of residual undeuterated solvent at δ 2.49 ppm (^{1}H) and 39.7 ppm (^{13}C), as the reference signals.

2.6 Physical Properties

2.6.1 Melting Points

Melting points were obtained on a Electrothermal IA 9200 series digital melting point apparatus (Department of Narcotics, Department of Medical Sciences).

2.6.2 Optical Rotations

Optical Rotations were measured on a Perkin Elmer 341 polarimeter using a sodium lamp operating at 589 nm (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.7 Solvents

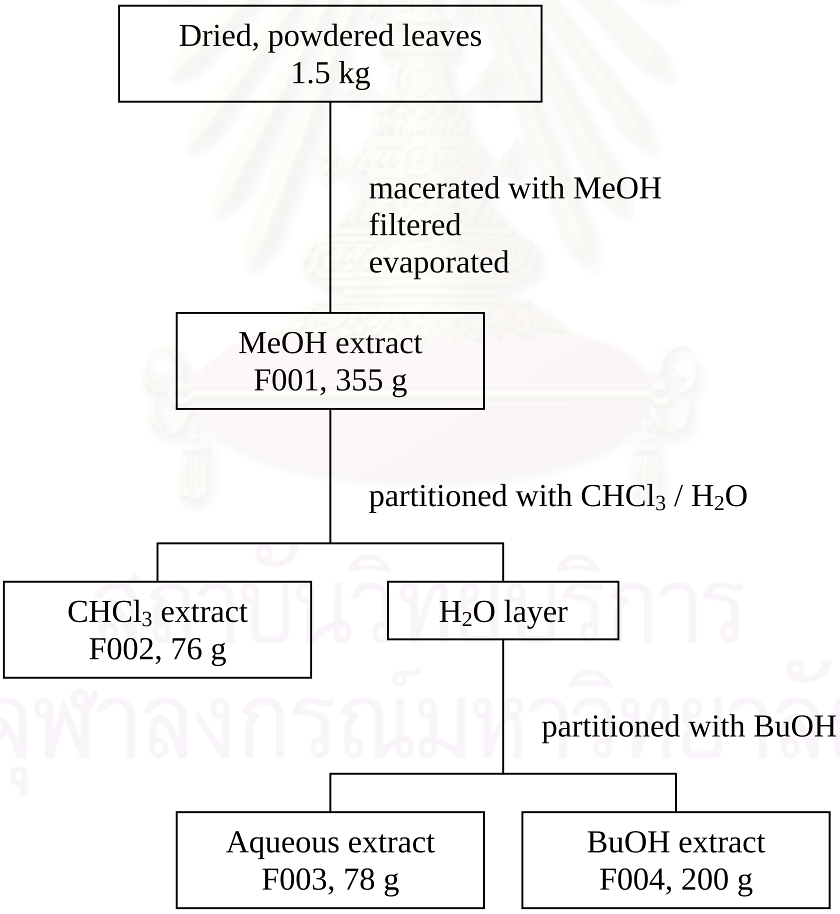
All organic solvents used in this work were commercial grade solvents, which were redistilled prior to use.

3. Extraction and isolation of *Ploiarium alternifolium* leaves.

3.1 Extraction

The dried, coarsely powdered leaves (1.5 kg) of *Ploiarium alternifolium* were marcerated with methanol (4×3 L) and filtered. The filtrates were evaporated under reduced pressure at temperature not exceeding 50 °C to yield 355 g of the methanol extract (F001).

The methanol extract was dissolved in 10% metanol in water and then partitioned with chloroform and butanol, respectively. Each extract was evaporated to give 76 g of the chloroform extract (F002), 78 g of the aqueous extract (F003), and 200 g of the butanol extract (F004) (Scheme 1).



Scheme 1 Extraction of *Ploiaruim alternifolium* leaves.

3.2 Isolation of the compounds from the butanol extract (F004)

TLC examination of the butanol extract showed several interesting yellowish, polar components. Therefore, it was further isolated by chromatographic techniques.

The butanol extract (F004, 100 g) was dissolved in a small amount of methanol, triturated with kieselguhr (diatomaceous earth) and dried. It was fractionated by quick column chromatography using a sintered glass filter column (17 cm inner diameter and 5 cm long) of silica gel. The organic phase of chloroform - methanol - water (5:6:4) was used as mobile phase for column and developing solvent for TLC. Each 300-ml fraction was collected and fractions with similar thin-layer chromatographic pattern were combined and evaporated to dryness, to give six fractions, F005-F010.

Table 3 Fractions obtained from the isolation of F004

Fraction	No. of eluate	Total weight (g)
F005	1-4	5.82
F006	5-16	14.33
F007	17-40	17.98
F008	41-44	2.12
F009	45-60	34.06
F010	61-66	8.29

Fraction F007 (17.8 g) was divided into two portions. Each portion was further isolated by a silica gel column (9.5 cm inner diameter and 6 cm long) using the same mobile phase as in quick column chromatography. Fractions of 50 ml were collected and similar ones were combined to yield eight fractions (F011-F018).

Table 4 Fractions obtained from the isolation of F007

Fraction	No. of eluate	Total weight (g)
F011	1-2	0.13
F012	3-4	0.14
F013	5-8	1.11
F014	9-18	3.42
F015	19-29	2.51
F016	30-60	2.04
F017	61-74	3.58
F018	75-83	2.53

Being examined by TLC, there were many yellowish polar components in the butanol extract. The suitable technique to isolate polar compounds without irreversible adsorption on solid support is high speed countercurrent chromatography (HSCCC), which is liquid-liquid chromatography.

3.2.1 Isolation of compounds PL-1 and PL-2 from F014

Fraction F014 (500 mg) was further chromatographed by HSCCC using the conditions below.

Sample : Fraction F014 (500 mg) was dissolved in equal volume (4 ml) of the aqueous and organic phase and filtered before injection.
Solvent system : Chloroform - methanol - water (5:10:6, v/v), isocratic
Stationary phase : Aqueous phase (upper phase)
Mobile phase : Organic phase (lower phase)
Mode : Normal phase ; LP, ORG, (H)→T
Rotational speed : 700 r.p.m.

Flow rate : 3 ml/min
Stationary phase
Fraction (S_F) : 0.61
Fraction volume 30 ml/fraction
Pressure : 75 psi

Fractions were examined by TLC using the mobile phase of HSCCC as the developing solvent. After combination of similar fractions, nine fractions were collected (F019-F027). Fraction F021 and fraction F024 yielded compounds PL-1 and PL-2, respectively (Scheme 2).

Table 5 Fractions obtained from the isolation of F014 (500 mg)

Fraction	No. of eluate	Total weight (mg)
F019	1-4	11
F020	5-8	56
F021	9-10	32
F022	11-13	38
F023	14-15	55
F024	16-17	67
F025	18-20	23
F026	21-24	35
F027	25-28	176

Fraction F021 (32 mg) gave yellow needle crystals (PL-1, 14 mg) which was washed with cool methanol and recrystallized from methanol. The TLC of purified crystal showed only one spot at R_f 0.50 (silica gel / organic phase of chloroform - methanol - water = 5:10:6). Compound PL-1 was identified as isoscutellarein 8-*O*-β-D-glucuronopyranoside methyl ester (Section 1, Chapter IV).

Fraction F024 (67 mg) gave yellow amorphous powder (PL-2, 30 mg). The TLC chromatogram of the purified compound showed a single spot at R_f 0.44

(silica gel / organic phase of chloroform - methanol - water = 5:10:6). Compound PL-2 was later identified as hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (Section 2, Chapter IV).

3.2.2 Isolation of compounds PL-3 and PL-4 from F013

Fraction F013 (870 mg) was divided into two portions. Each portion (550 and 320 mg) was further isolated by HSCCC using the conditions below.

Sample : Each portion was dissolved in equal volume (4 ml) of the aqueous and organic phase and filtered before injection.
Solvent system : Chloroform - methanol - water (7:13:8, v/v), isocratic
Stationary phase : Aqueous phase (upper phase)
Mobile phase : Organic phase (lower phase)
Mode : Normal phase ; LP, ORG, (H)→T
Rotational speed : 760 r.p.m.
Flow rate : 3 ml/min
Stationary phase
Fraction (S_F) : 0.66
Fraction volume : 30 ml/fraction :
Pressure 75-100 psi

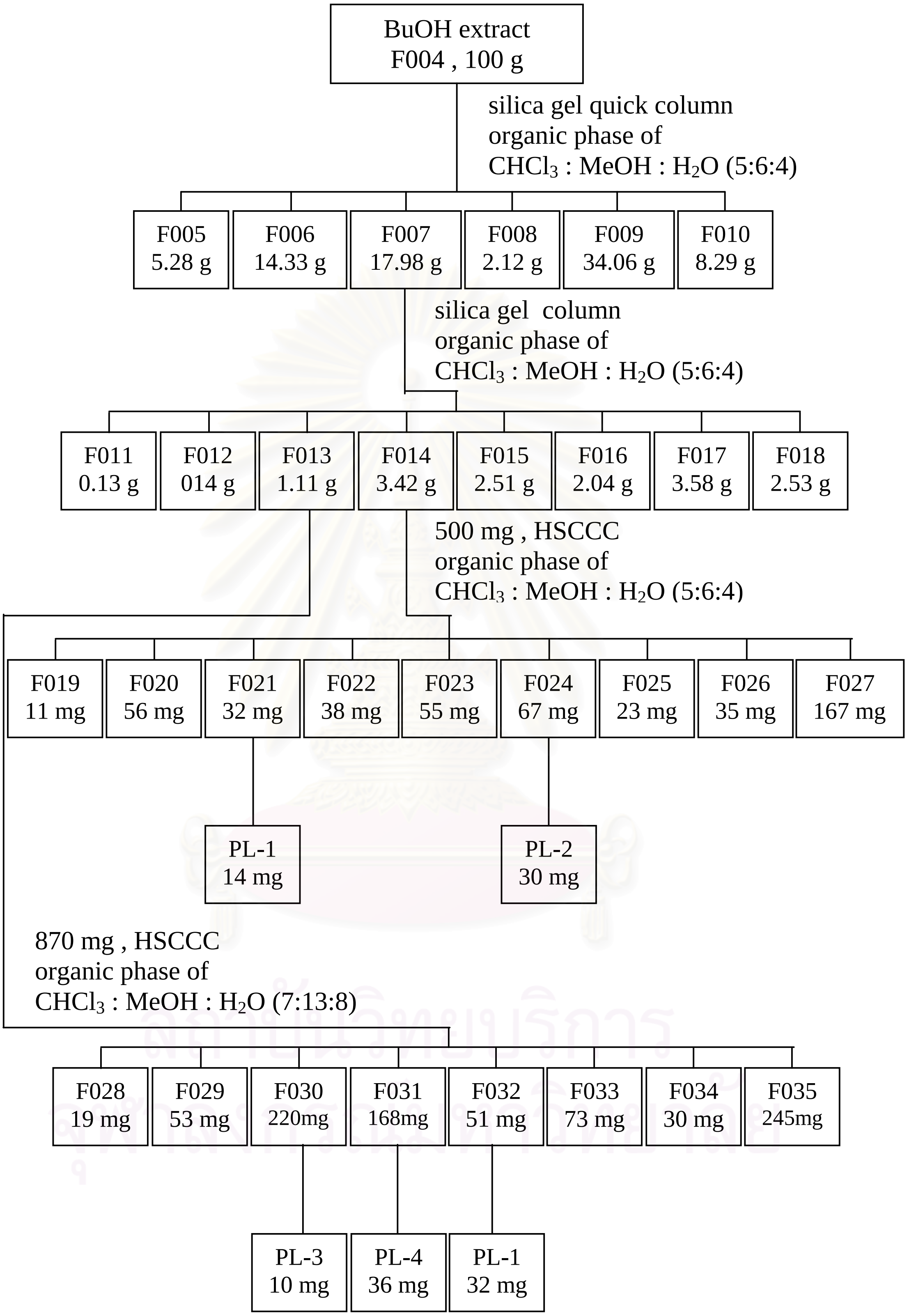
Similar fractions, examined by TLC using the mobile phase of HSCCC as the developing solvent, were combined to yield eight fractions (F028-F035). This separation provided compounds PL-3 and PL-4 from fraction F030 and fraction F031, respectively (Scheme 2). Moreover, fraction F032 also gave compound PL-1 (32 mg) which was formerly obtained from fraction F021.

Table 6 Fractions obtained from the isolation of F013 (870 mg)

Fraction	No. of eluate	Total weight (mg)
F028	1-4	19
F029	5-8	53
F030	9-10	220
F031	11-13	168
F032	14-16	51
F033	17-22	73
F034	23-25	30
F035	26-34	245

Fraction F030 (220mg) was crystallized from methanol to yield 10 mg of yellow needle crystals (PL-3). The TLC of purified compound showed only single spot at R_f 0.53 (silica gel / organic phase of chloroform - methanol - water = 7:13:8) that was later identified as isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (Section 3, Chapter IV).

Fraction F031 (168 mg) gave yellow amorphous powder (PL-4, 36 mg) which precipitated from methanol. The TLC of purified compound showed one spot at R_f 0.47 (silica gel / organic phase of chloroform - methanol - water = 7:13:8). Compound PL-4 was subsequently identified as hypolaetin 8-*O*-*β*-D- glucuronopyranoside butyl ester (Section 4, Chapter IV).



Scheme 2 Fractionation of the butanol extract from *Ploiarium alternifolium* leaves

4. Physical and chemical properties of the isolated compounds

4.1 Compound PL-1

M.P. : 260 – 263 ^{o}C

$[\alpha]^{25}_{D}$: + 147.10 ^{o} (c 0.104, MeOH)

UV : λ_{max} (MeOH) nm (log ε); Figure 10
272(4.19), 334(4.16)
: λ_{max} (MeOH + NaOH) nm (log ε); Figure 11
279(4.25), 326(3.96), 399(4.37)
: λ_{max} (MeOH + $AlCl_3$) nm (log ε); Figure 12
278(4.12), 306(4.10), 349(4.20), 389(4.06)
: λ_{max} (MeOH + $AlCl_3$ + HCl) nm (log ε); Figure 13
229(4.16), 280(4.11), 306(4.12), 344(4.18), 388(3.97)
: λ_{max} (MeOH + NaOAc) nm (log ε); Figure 14
280(4.36), 363(4.04)
: λ_{max} (MeOH + NaOAc + H_3BO_3) nm (log ε) ; Figure 15
278(4.27), 350(4.14)

IR : ν_{max} cm^{-1}, KBr disc; Figure 16
3445, 3104, 1732, 1658, 1554, 1453

ESI-TOF MS : m/z; Figure 17
499 [M+Na]$^+$, 477 [M+H]$^+$

^{1}H NMR : δ_H (ppm), 300 MHz, in DMSO-d_6 ; Figure 18
3.0-3.5 (3H , H-2″, H-3″, and H-4″), 3.57 (3H, s, OMe), 3.91 (1H, d, J = 9.6 Hz, H-5″), 4.81 (1H, d, J = 7.8 Hz, H-1″), 6.25 (1H, s, H-6), 6.77 (1H, s, H-3), 6.88 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 8.00 (2H, d, J = 8.6 Hz, H-2′ and H-6′), 12.76 (1H, s, 5- OH)

^{13}C NMR : δ_C (ppm), 75 MHz, in DMSO-d_6 ; Figure 19
51.9 (q, OMe), 71.5 (d, C-4″), 73.8 (d, C-2″), 75.1 (d, C-3″), 75.6 (d, C-5″), 98.9 (d, C-6), 102.3 (d, C-3), 103.2 (s, C-4a), 106.3 (d, C-1″), 115.7 (d, C-3′ and C-5′), 121.0 (s, C-1′), 125.1 (s, C-8), 128.6 (d, C-2′ and C-6′), 149.1 (s, C-8a),

157.1 (s, C-5 and C-7), 160.9 (s, C-4′), 163.5 (s, C-2), 169.1 (s, C-6″), 181.5 (s, C-4)

4.2 Compound PL-2

M.P. : 246 – 249 ^{o}C
$[\alpha]^{25}_{D}$: + 146.15 ^{o} (c 0.118, MeOH)
UV : λ_{max} (MeOH) nm (log ε); Figure 29
271(4.17), 352(4.20)
: λ_{max} (MeOH + NaOH) nm (log ε); Figure 30
275(4.28), 413(4.34)
: λ_{max} (MeOH + $AlCl_3$) nm (log ε); Figure 31
276(4.30), 431(4.38)
: λ_{max} (MeOH + $AlCl_3$ + HCl) nm (log ε); Figure 32
275(4.14), 299(3.97), 360(4.13)
: λ_{max} (MeOH + NaOAc) nm (log ε); Figure 33
280(4.30), 326(4.06), 372(4.09)
: λ_{max} (MeOH + NaOAc + H_3BO_3) nm (log ε); Figure 34
263(4.28), 379(4.27)
IR : ν_{max} cm^{-1}, KBr disc; Figure 35
3335, 3173, 1728, 1658, 1571, 1452
ESI-TOF MS : m/z; Figure 36
515 $[M+Na]^+$, 493 $[M+H]^+$
^{1}H NMR : δ_H (ppm), 300 MHz, in DMSO-d_6 ; Figures 37 - 38
3.0-3.5 (3H, H-2″, H-3″, and H-4″), 3.55 (3H, s, OMe), 3.89 (1H, d, *J* = 9.6 Hz, H-5″), 4.81 (1H, d, *J* = 7.7 Hz, H-1″), 6.25 (1H, s, H-6), 6.63(1H, s, H-3), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 7.38 (1H, s, H-2′), 7.56 (1H, d, J = 8.4 Hz, H-6′), 12.80 (1H, s, 5-OH)
^{13}C NMR : δ_C (ppm), 75 MHz, in DMSO-d_6 ; Figure 39
51.9 (q, OMe), 71.4 (d, C-4″), 73.7 (d, C-2″), 75.1 (d, C-3″), 75.6 (d, C-5″), 98.9 (d, C-6), 102.3 (d, C-3), 103.3 (s, C-4a),

106.1 (d, C-1″), 113.6 (d, C-2′), 115.7 (d, C-5′), 119.4 (d, C-6′), 121.3 (s, C-1′), 124.9 (s, C-8), 145.4 (s, C-3′), 149.2 (s, C-4′), 149.7 (s, C-8a), 157.1 (s, C-5 and C-7), 163.8 (s, C-2), 168.2 (s, C-6″), 181.5 (s, C-4)

4.3 Compound PL-3

M.P. : 255 – 257 °C
$[\alpha]^{25}_{D}$: + 118.17 ° (c 0.100, MeOH)
UV : λ_{max} (MeOH) nm (log ε); Figure 49
277(4.35), 302(4.25), 345(4.19)
: λ_{max} (MeOH + NaOH) nm (log ε)
279(4.40), 399(4.49)
: λ_{max} (MeOH + $AlCl_3$) nm (log ε)
279(4.27), 306(4.24), 349(433), 390(4.16)
: λ_{max} (MeOH + $AlCl_3$ + HCl) nm (log ε)
280(4.27), 306(4.26), 344(4.32), 389(4.08)
: λ_{max} (MeOH + NaOAc) nm (log ε)
280(4.48), 372(4.15)
: λ_{max} (MeOH + NaOAc + H_3BO_3) nm (log ε)
280(4.47), 314(4.38), 351(4.43)
IR : ν_{max} cm^{-1}, KBr disc; Figure 50
3158(br), 2925, 1727, 1654, 1578, 1446
FABMS : m/z; Figure 51
519 $[M+H]^+$
^{1}H NMR : δ_H (ppm), 300 MHz, in DMSO-d_6 ; Figure 52

0.73 (3H, t, *J* = 7.3 Hz, H-4‴), 1.14 (2H, sextet, *J* = 7.3 Hz, H-3‴), 1.36 (2H, m, H-2‴), 3.0-3.5 (3H, H-2″, H-3″, and H-4″), 3.87 (1H, d, *J* = 9.7 Hz, H-5″), 3.97 (2H, dd, *J* = 6.5, 6.3 Hz, H-1‴), 4.80 (1H, d, *J* = 7.8 Hz, H-1″), 6.24 (1H, s, H-6), 6.81(1H, s, H-3), 6.87 (2H, d, *J* = 8.6 Hz, H-3′ and H-5′), 8.00 (2H, d, *J* = 8.6 Hz, H-2′ and H-6′), 12.78 (1H, s, 5-OH)

^{13}C NMR : δ_C (ppm), 75 MHz, in DMSO-d_6 ; Figure 53
13.5 (q, C-4‴), 18.5 (t, C-3‴), 30.0 (t, C-2‴), 64.3 (t, C-1‴), 71.4 (d, C-4″), 73.8 (d, C-2″), 75.1 (d, C-3″), 75.6 (d, C-5″), 98.9 (d, C-6), 102.2 (d, C-3), 103.1 (s, C-4a), 106.3 (d, C-1″), 115.7 (d, C-3′ and C-5′), 121.0 (s, C-1′), 125.0 (s, C-8), 128.6 (d, C-2′ and C-6′), 149.1 (s, C-8a), 157.1 (s, C-7), 157.2 (s, C-5), 161.0 (s, C-4′), 163.5 (s, C-2), 168.6 (s, C-6″), 181.5 (s, C-4)

4.4 Compound PL-4

M.P. : 259 – 262 °C

$[\alpha]^{25}_{D}$: + 126.90 ° (c 0.100, MeOH)

UV : λ_{max} (MeOH) nm (log ε); Figure 61
272(4.23), 355(4.20)
: λ_{max} (MeOH + NaOH) nm (log ε)
271(4.33), 412(4.36)
: λ_{max} (MeOH + $AlCl_3$) nm (log ε)
276(4.31), 423(4.30)
: λ_{max} (MeOH + $AlCl_3$ + HCl) nm (log ε)
276(4.21), 301(4.04), 356(4.19)
: λ_{max} (MeOH + NaOAc) nm (log ε)
280(4.37), 380(4.22)
: λ_{max} (MeOH + NaOAc + H_3BO_3) nm (log ε)
269(4.43), 380(4.37)

IR : ν_{max} cm^{-1}, KBr disc; Figure 62
3071, 2738, 1739, 1656, 1576

FABMS : m/z; Figure 63
535 $[M+H]^+$

^{1}H NMR : δ_H (ppm), 300 MHz, in DMSO-d_6 ; Figures 64-65
0.74 (3H, t, *J* = 7.3 Hz, H-4‴), 1.14 (2H, sextet, *J* = 7.3 Hz, H-3‴), 1.35 (2H, m, H-2‴), 3.0-3.5 (3H, H-2″, H-3″, and H-

4″), 3.85 (1H, d, *J* = 9.7 Hz, H-5″), 3.95 (2H, dd, *J* = 6.2, 5.6 Hz, H-1‴), 4.81 (1H, d, *J* = 7.8 Hz, H-1″), 6.26 (1H, s, H-6), 6.63(1H, s, H-3), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 7.38 (1H, d, *J* = 1.9 Hz, H-2′), 7.56 (1H, dd, *J* = 8.4, 1.9 Hz, H-6′), 12.79 (1H, s, 5-OH)

^{13}C NMR : δ_C (ppm), 75 MHz, in DMSO-d_6 ; Figure 66

13.5 (q, C-4‴), 18.4 (t, C-3‴), 30.0 (t, C-2‴), 64.4 (t, C-1‴), 71.4 (d, C-4″), 73.8 (d, C-2″), 75.3 (d, C-3″), 75.7 (d, C-5″), 99.0 (d, C-6), 102.4 (d, C-3), 103.5 (s, C-4a), 106.0 (d, C-1″), 113.7 (d, C-2′), 115.8 (d, C-5′), 119.6 (d, C-6′), 121.5 (s, C-1′), 125.0(s, C-8), 145.7 (s, C-3′), 149.5 (s, C-4′), 150.0 (s, C-8a), 156.9 (s, C-7), 157.4 (s, C-5), 164.1 (s, C-2), 168.8 (s, C-6″), 181.9 (s, C-4)

CHAPTER IV

RESULTS AND DISCUSSION

The leaves of *Ploiarium alternifolium* (1.5 kg) were macerated with methanol and the methanol extract was partitioned with chloroform and butanol, respectively. When examined by TLC, the butanol extract showed several interesting, yellowish polar components. Therefore, it was further separated by chromatographic techniques, mainly using the high speed countercurrent chromatography(HSCCC) to afford four pure compounds.

The structures of these isolated compounds were determined by analyses of their spectroscopic data, including UV, IR, MS and NMR spectral data, and then confirmed by comparison with literature.

1.Structure elucidation of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester

Compound PL-1 was obtained as yellow-needle crystals showing optical rotation $[\alpha]^{25}{}_{D}$ of + 147.10° (c0.104, MeOH). The UV spectrum in MeOH (Figure 10) exhibited λ_{max} at 272, 334 nm. The IR spectrum (Figure 16) displayed characteristic bands at 3445, 3104 (hydroxyl group), 1732 (ester carbonyl), 1658 (ketone carbonyl) and 1554, 1453 (aromatic ring). The ESI-TOF mass spectrum (Figure 17) of this compound showed the pseudomolecular ion peak at m/z 499 $[M+Na]^+$ and m/z 477 $[M+H]^+$ implying the molecular formula of $C_{22}H_{20}O_{12}$.

The 300 MHz ^{1}H NMR spectrum in DMSO-d_6 of compound PL-1 (Figure 18) exhibited one methyl proton signal at δ 3.57 ppm ; five methine proton signals at δ 3.0-3.5 (3H), 3.91, and 4.81 ppm ; six aromatic and olefinic proton signals at δ 6.25, 6.77, 6.88 (2H), and 8.00 (2H) ppm ; and a chelated hydroxyl signal at δ 12.76 ppm. The 75 MHz ^{13}C NMR spectrum in DMSO-d_6 (Figure 19) indicated twenty-two carbons, consistent with the molecular formula. The carbon signals were classified by the DEPT 135 (Figure 20) and the HMQC spectra (Figures 21-22) as one methyl carbon signal at δ 51.9 ppm ; five oxymethine carbon signals at δ 71.5, 73.8, 75.1, 75.6 and 106.3 ppm ; six aromatic and olefinic carbon signals at δ 98.9, 102.3, 115.7 (2C), and 128.6 (2C) ppm ; and ten quaternary carbon signals at δ 103.2, 121.0, 125.1, 149.1, 157.1 (2C), 160.9, 163.5, 169.1, and 181.5 ppm. Analyses of the ^{13}C NMR (Table 7) indicated the presence of an ester carbonyl carbon at δ 169.1 ppm and a ketone carbonyl at δ 181.5 ppm that were assigned as C-6″ and C-4, respectively.

From the ^{1}H NMR and ^{13}C NMR spectral data, the most downfield proton signal was at δ 12.76 ppm, suggesting the presence of the chelated hydroxyl group in compound PL-1. Thus the proton signal was assigned to 5-OH which chelated with the ketone carbonyl at C-4. The aromatic proton signals at δ 6.88 (2H) and 8.00 (2H) ppm were the two equivalent sets of the ortho-coupled proton signals (J = 8.6 Hz). These proton signals were assigned to H-2′, H-6′ (δ 8.00 ppm) and H-3′, H-5′(δ 6.88 ppm) of ring B. So 4′-position should be substituted with a hydroxyl group. The other singlet proton signals at δ 6.77 and 6.25 ppm were assigned as H-3 and H-6, respectively. Therefore, a hydroxyl group should be substituted at 7-position. In the ^{13}C NMR spectrum, the upfield signal region (δ 71.5-75.6 ppm) indicated the presence of a sugar part. The anomeric carbon signal (δ 106.3 ppm) and the anomeric proton signal (δ 4.81 ppm) also suggested compound PL-1 as having one sugar moiety.

Compound PL-1, therefore, has two major parts, one flavone aglycone and one sugar moiety. The ^{1}H-^{1}H COSY spectrum (Figures 23-24) exhibited the proton connectivities in the aglycone and sugar moiety as follow : H-2′ or H-6′ / H-3′ or H-5′ ; H-1″ / H-2″ ; H-4″ / H-5″ (Figure 2).

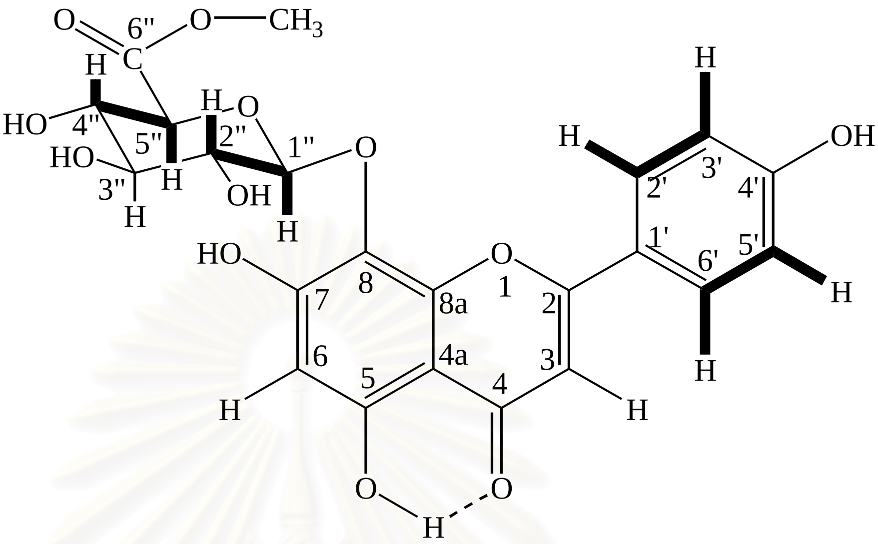


Figure 2 The ^{1}H-^{1}H correlations (bold line) in the ^{1}H-^{1}H COSY spectrum of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

The complete assignments of compound PL-1 were achieved by analysis of the HMBC ($^{n}J_{HC}$ = 8 and 4 Hz) spectra (Figures 25-28). The HMBC spectra exhibited the ^{1}H-^{13}C long-range correlations in the aglycone as follows : the correlations of 5-OH (δ 12.76 ppm) to C-6 (δ 98.9 ppm) and the correlations of H-6 (δ 6.25 ppm) to C-5 (δ 157.1 ppm), C-7 (δ 157.1 ppm) supported the positions of the hydroxyl groups at 5-position and 7-position. The correlations of H-2′, H-6′ [δ 8.00 ppm (2H)] and H-3′, H-5′ [δ 6.88 ppm (2H)] to C-4′ (δ 160.9 ppm) confirmed that the hydroxyl group was located at 4′-position. The connection between rings A and C was exhibited by the correlations of H-3 (δ 6.77 ppm) and H-6 (δ 6.25 ppm) to C-4a (δ 103.2 ppm). The linkage between ring B and ring C was assigned by the correlations of H-2′, H-6′[δ 8.00 ppm (2H)] to C-2 (δ 163.5 ppm) and H-3 (δ 6.77 ppm) to C-1′ (δ 121.0 ppm).

For the sugar moiety, the anomeric proton (δ 4.81 ppm) having the correlation with C-8 (δ 125.1 ppm) indicated 8-*O*-substitution of the flavone aglycone. The *J* value (7.8 Hz) of the doublet of anomeric proton suggested the *β*-configuration of the sugar. The sugar moiety was then assigned as 8-*O*-*β*-D-glucuronopyranoside methyl ester by the correlation of COOC<u>H</u>$_3$ (δ 3.57 ppm, 3H) to C-6″ (δ 169.1 ppm). The ^{1}H-^{13}C long-range correlations from the HMBC spectrum of compound PL-1 in DMSO-d_6 are shown in Figure 3 and summarized in Table 8.

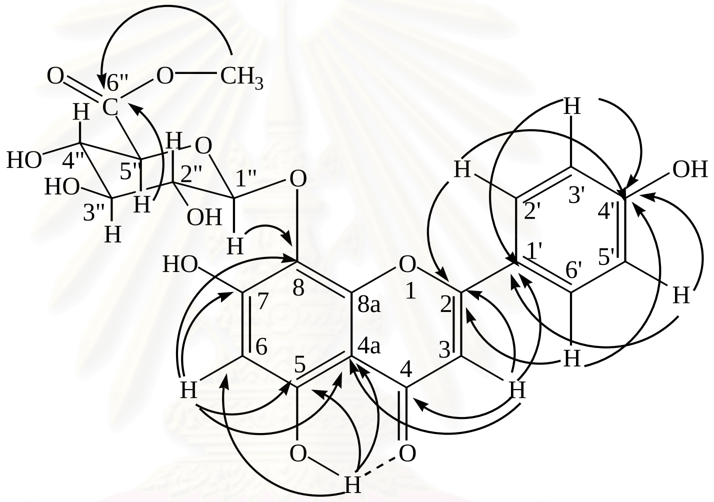


Figure 3 The ^{1}H-^{13}C long-range correlations in the HMBC spectrum of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

The spectral data of compound PL-1 were compared with those of the known compound, isoscutellarein 8-*O*-*β*-D-glucuronopyranoside (Billeter, Meier, and Sticher, 1991). The data are shown in Table 8. Compound PL-1 was similar to the known compound except the presence of carboxymethyl group at δ_H 3.57 ppm and δ_C 51.9 ppm. Therefore, compound PL-1 was determined as isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester.

Table 7 The ^{1}H -^{13}C NMR and HMBC spectral data of PL-1 in DMSO-d_6

Position	PL-1			
	δ_C (ppm)	δ_H (ppm), mult. (*J* in Hz)	HMBC correlations	
			$^{n}J_{HC}$ = 8 Hz	$^{n}J_{HC}$ = 4 Hz
Aglycone				
2	163.5	-	-	-
3	102.3	6.77, s	C-2, C-4, C-1′	C-2, C-4a
4	181.5	-	-	-
4a	103.2	-	-	-
5	157.1	12.76, OH, s	C-4a, C-5, C-6	C-4a, C-5, C-6
6	98.9	6.25, s	C-4a, C-5, C-7, C-8	C-4a, C-5, C-7, C-8
7	157.1	-	-	-
8	125.1	-	-	-
8a	149.1	-	-	-
1′	121.0	-	-	-
2′	128.6	8.00, d (8.6)	C-2, C-4′	C-2, C-4′
3′	115.7	6.88, d (8.6)	C-1′, C-4′	C-4′
4′	160.9	-	-	-
5′	115.7	6.88, d (8.6)	C-1′, C-4′	C-4′
6′	128.6	8.00, d (8.6)	C-2, C-4′	C-2, C-4′
Sugar				
1″	106.3	4.81, d (7.8)	C-8	C-8
2″	73.8	3.0 – 3.5	-	-
3″	75.1	3.0 – 3.5	-	-
4″	71.5	3.0 – 3.5	-	-
5″	75.6	3.91, d (9.6)	C-6″	C-6″
6″	169.1	-	-	-
$COO\underline{C}H_3$	51.9	3.57, 3H, s	C-6″	C-6″

Table 8 The ^{1}H-^{13}C NMR spectral data of PL-1 (in DMSO-d_6) and isoscutellarein 8-*O*-*β*-D-glucuronopyranoside (in DMSO-d_6)

Position	PL-1		isoscutellarein 8-*O*-*β*-D-glucuronopyranoside[a]
	δ_C (ppm)	δ_H (ppm), mult. (*J* in Hz)	δ_C (ppm)
Aglycone			
2	163.5	-	163.8
3	102.3	6.77, s	102.3
4	181.5	-	181.7
4a	103.2	-	103.3
5	157.1	12.76, OH, s	157.2
6	98.9	6.25, s	99.0
7	157.1	-	157.2
8	125.1	-	125.2
8a	149.1	-	149.2
1′	121.0	-	121.0
2′	128.6	8.00, d (8.6)	128.9
3′	115.7	6.88, d (8.6)	116.0
4′	160.9	-	161.1
5′	115.7	6.88, d (8.6)	116.0
6′	128.6	8.00, d (8.6)	128.9
Sugar			
1″	106.3	4.81, d (7.8)	106.3
2″	73.8	3.0 – 3.5	73.6
3″	75.1	3.0 – 3.5	76.1
4″	71.5	3.0 – 3.5	71.5
5″	75.6	3.91, d (9.6)	75.3
6″	169.1	-	170.1
$COO\underline{C}H_3$	51.9	3.57, 3H, S	-

[a] reported by Billeter *et al*., 1991

2. Structure elucidation of hypolaetin 8-*O*-β-D-glucuronopyranoside methyl ester (PL-2)

Compound PL-2 was isolated as yellow amorphous powder. The UV spectrum in MeOH (Figure 29) displayed λ_{max} at 271 and 352 nm. The IR spectrum (Figure 35) exhibited absorption bands at 3335, 3173 (hydroxyl group), 1728 (ester carbonyl), 1658 (ketone carbonyl) and 1571, 1452 cm^{-1} (aromatic ring). The ESI-TOF mass spectrum (Figure 36) established the molecular formula of this compound as $C_{22}H_{20}O_{13}$, showing the pseudomolecular ion peak at m/z 493 $[M+H]^+$ and 515 $[M+Na]^+$. This compound showed optical rotation $[\alpha]^{25}_{D}$ of + 146.15 ° (c 0.118, MeOH).

The 300 MHz ^1H NMR spectrum in DMSO-d_6 of compound PL-2 (Figures 37-38) suggested that this compound contained one methyl ester proton appearing as the signal at δ 3.55 ppm ; five methine proton signals at δ 3.0-3.5 (3H), 3.89, and 4.81 ppm ; five aromatic and olefinic proton signals at δ 6.25, 6.63, 6.86, 7.38, and 7.56 ppm ; and a chelated hydroxyl at δ 12.80 ppm. The 75 MHz ^{13}C NMR spectrum in DMSO-d_6 (Figure 39) showed twenty-two carbons. These carbon signals were classified by the DEPT 135 (Figure 40) and the HMQC spectra (Figures 41-42) as one methyl ester carbon signal at δ 51.9 ppm ; five oxymethine carbon signals at δ 71.4, 73.7, 75.1, 75.6 and 106.1 ppm ; five aromatic and olefinic carbon signals at δ 98.9, 102.3, 113.6, 115.7, and 119.4 ppm ; and eleven quaternary carbon signals at δ 103.3, 121.3, 124.9, 145.4, 149.2, 149.7, 157.1 (2C), 163.8, 168.2, and 181.5 ppm.

Most of the proton and carbon resonances in the ^1H and ^{13}C NMR spectra of compounds PL-1 (isoscutellarein 8-*O*-β-D-glucuronopyranoside methyl ester) and PL-2 were similar except for the resonances in ring B. For compound PL-2, one set of ortho-coupled protons (*J* = 8.4 Hz) at δ 6.86 and 7.56 ppm, and one singlet proton signal at δ 7.38 ppm were presented. This evidence indicated that ring B of compound PL-2 should be substituted with more hydroxyl group than in compound PL-1. There were two possibilities for the two hydroxyl groups in ring B: one was 2′, 4′-dihydroxyl, whereas the other was 3′, 4′-dihydroxyl substitution. In the case of 2′, 4′-dihydroxyl substitution, the aromatic carbon signal at 3′-position would have been more the upfield similar to the carbon at 6-position (δ_C 98.9 ppm) due to the influence of dihydroxyl groups between 2′-position and 4′-position. However, in this case, no such upfield aromatic carbon signal was detected. In addition, the UV absorption maximum at 352 nm was bathochromically shifted with AlCl$_3$ and NaOAc/H$_3$BO$_3$, indicative of a flavone having an ortho dihydroxyl moiety in the structure.

The ^{1}H-^{1}H COSY spectrum of compound PL-2 (Figures 43-44) revealed the similarity of its sugar moiety to that of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (compound PL-1). The observed ^{1}H-^{1}H correlations were indicative of the proton connectivities as follows: H-2′ / H-6′ / H-5′ ; H-1″ / H-2″ ; H-4″ / H-5″ (Figure 4).

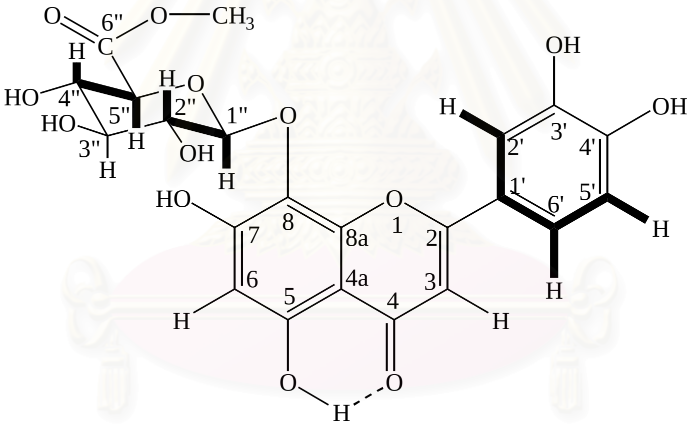


Figure 4 The ^{1}H-^{1}H correlations (bold line) in the ^{1}H-^{1}H COSY spectrum of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2).

The complete assignments of compound PL-2 were achieved by analysis of the HMBC (${}^{n}J_{HC}$ = 8 and 4 Hz) spectra (Figures 45-48). The ${}^{1}H$-${}^{13}C$ long-range correlations from the HMBC spectrum of compound PL-2 in DMSO-d_6 are shown in Figure 5 and summarized in Table 9.

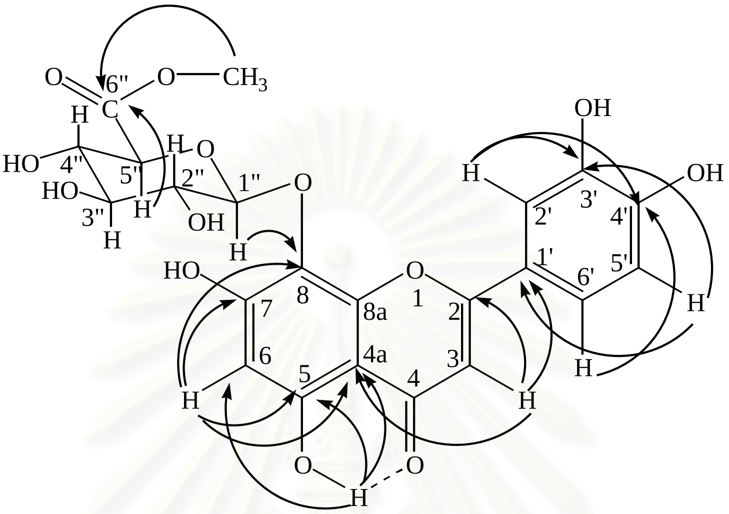


Figure 5 The ${}^{1}H$-${}^{13}C$ long-range correlations in the HMBC spectrum of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

Comparison of ${}^{1}H$ and ${}^{13}C$ NMR spectral data of compound PL-2 with those of hypolaetin 8-*O*-*β*-D-glucuronopyranoside (Billeter *et al.*, 1991) is shown in Table 10. Compound PL-2 appeared to be similar to the known compound except for the additional presence of carboxymethyl group at δ_H 3.55 ppm and δ_C 51.9 ppm. Therefore, the structure of compound PL-2 was identified as hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester.

Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of PL-2 in DMSO-d_6

Position	PL-2			
	δ_C (ppm)	δ_H (ppm), mult. (*J* in Hz)	HMBC correlations	
			$^nJ_{HC}$ = 8 Hz	$^nJ_{HC}$ = 4 Hz
Aglycone				
2	163.8	-	-	-
3	102.3	6.63, s	C-2, C-4a	C-2, C-4a
4	181.5	-	-	-
4a	103.3	-	-	-
5	157.1	12.80, OH, s	C-4a, C-5, C-6	C-4a, C-5, C-6
6	98.9	6.25, s	C-4a, C-5, C-7, C-8	C-4a, C-5, C-7, C-8
7	157.1	-	-	-
8	124.9	-	-	-
8a	149.7	-	-	-
1′	121.3	-	-	-
2′	113.6	7.38, br s	C-2, C-4′, C-6′	C-2, C-3′, C-4′
3′	145.4	-	-	-
4′	149.2	-	-	-
5′	115.7	6.86, d (8.4)	C-1′, C-3′	C-3′
6′	119.4	7.56, br d (8.4)	-	C-4′
Sugar				
1″	106.1	4.81, d (7.7)	C-8	C-8
2″	73.7	3.0 – 3.5	-	-
3″	75.1	3.0 – 3.5	-	-
4″	71.4	3.0 – 3.5	-	-
5″	75.6	3.89, d (9.6)	C-6″	C-6″
6″	168.2	-	-	-
COO$\underline{C}$H$_3$	51.9	3.55, 3H, s	C-6″	C-6″

Table 10 The ^{1}H and ^{13}C NMR spectral data of PL-2 (in DMSO-d_6) and hypolactin 8-*O*-β-D-glucuronopyranoside (in DMSO-d_6)

Position	PL-2		hypolaetin 8-*O*-β-D-glucuronopyranoside [a]
	δ_C (ppm)	δ_H (ppm), mult. (J in Hz)	δ_C (ppm)
Aglycone			
2	163.8	-	164.7
3	102.3	6.63, s	102.6
4	181.5	-	181.3
4a	103.3	-	102.5
5	157.1	12.80, OH, s	157.3
6	98.9	6.25, s	99.5
7	157.1	-	159.1
8	124.9	-	126.1
8a	149.7	-	149.3
1′	121.3	-	121.6
2′	113.6	7.38, br s	114.0
3′	145.4	-	146.5
4′	149.2	-	149.6
5′	115.7	6.86, d (8.4)	115.6
6′	119.4	7.56, br d (8.4)	118.5
Sugar			
1″	106.1	4.81, d (7.7)	107.3
2″	73.7	3.0 – 3.5	74.1
3″	75.1	3.0 – 3.5	76.5
4″	71.4	3.0 – 3.5	71.8
5″	75.6	3.89, d (9.6)	75.7
6″	168.2	-	172.0
COO$\underline{C}H_3$	51.9	3.55, 3H, s	-

[a] reported by Billeter *et al.*, 1991

3. Structure elucidation of isoscutellarein 8-*O*-β-D-glucuronopyranoside butyl ester (PL-3)

Compound PL-3 was isolated as yellow-needle crystals showing optical rotation $[\alpha]^{25}{}_{D}$ of + 118.17 (c 0.100, MeOH). The UV spectrum in MeOH (Figure 49) showed λ_{max} at 277 and 302 nm. The IR spectrum (Figure 50) exhibited absorption bands at 3158 (hydroxyl group), 2925 (aliphatic), 1727 (ester carbonyl), 1654 (ketone carbonyl), and 1578, 1446 cm^{-1} (aromatic ring). The FABMS spectrum (Figure 51) of this compound showed the pseudomolecular ion peak at m/z 519 $[M+H]^+$, implying the molecular formula of $C_{25}H_{26}O_{12}$.

The 300 MHz ^{1}H NMR spectrum of compound PL-3 in DMSO-d_6 (Figure 52) exhibited one methyl proton signal at δ 0.73 ppm ; three methylene proton signals at δ 1.14, 1.36, and 3.97 ppm ; five oxymethine proton signals at δ 3.0-3.5 (3H), 3.87, and 4.80 ppm ; six aromatic and olefenic proton signals at δ 6.24, 6.81, 6.87 (2H), and 8.00 (2H) ppm ; and a chelated hydroxyl proton at δ 12.78 ppm. The 75 MHz ^{13}C NMR spectrum in DMSO-d_6 (Figure 53) showed signals for twenty-five carbons, which were classified by the DEPT 135 spectrum (Figure 54) as one methyl carbon signal at δ 13.5 ppm ; three methylene carbon signals at δ 18.5, 30.0, and 64.3 ppm ; five oxymethine carbon signals at δ 71.4, 73.8, 75.1, 75.6 and 106.3 ppm ; six aromatic and olefinic carbon signals at δ 98.9, 102.2, 115.7 (2C), and 128.6 (2C) ppm ; and ten quaternary carbon signal at δ 103.1, 121.0, 125.0, 149.1, 157.1, 157.2, 161.0, 163.5, 168.6, and 181.5 ppm. Further assignments of the directly bonded protons and carbons by analyses of the HMQC spectrum (Figure 55) are shown in Table 11.

The ^{1}H and ^{13}C NMR spectra of compound PL-3 were similar to those of isoscutellarein 8-*O*-β-D-glucuronopyranoside (Billeter *et al.,* 1991). However, both the additional proton signals [one methyl proton (δ 0.73 ppm) and three methylene protons (δ 1.14, 1.36, and 3.97 ppm)] and carbon signals [one methyl carbon (δ 13.5 ppm) and three methylene carbon (δ 18.5, 30.0, and 64.3 ppm)] could be clearly observed on the NMR spectral data of compound PL-3.

Analysis of the ^1H-^1H COSY (Figure 56) and the HMQC spectra led to the assignments of these additional protons and carbons as parts of a butyl fragment. The ^1H-^1H COSY connectivities of compound PL-3 were as follows : H-2′ or H-6′ / H-3′ or H-5′ ; H-1″ / H-2″ ; H-4″ / H-5″ ; H-1‴ / H-2‴ / H-3‴ / H-4‴ (Figure 6).

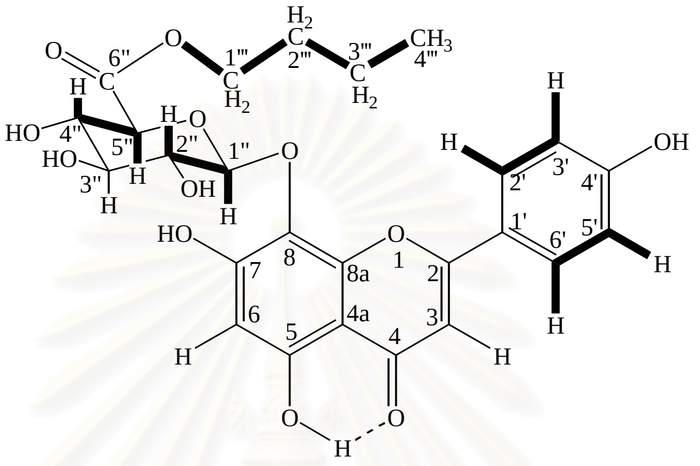


Figure 6 The ^1H-^1H correlation (bold line) in the ^1H-^1H COSY spectrum of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3).

The HMBC spectra ($^nJ_{HC}$ = 8 and 4 Hz) of compound PL-3 (Figures 57-60)supported the presence of butyl fragment by showing the correlations of H_2-1‴ (δ 3.97 ppm) to C-3‴ (δ 18.5 ppm); H_2-3‴(δ 1.14 ppm) to C-2‴ (δ 30.0 ppm) and C-4‴ (δ 13.5 ppm); and H_3-4‴ (δ 0.73 ppm) to C-2‴ and C-3‴. This fragment was assigned to be butyl ester group of the sugar moiety according to the correlation between H-1‴ (δ 3.97 ppm) and C-6″ (δ 168.6 ppm). Therefore, the sugar moiety was 8-*O*-*β*-D-glucuronopyranoside butyl ester. The ^1H-^{13}C long-range HMBC correlations of compound PL-3 in DMSO-d_6 are shown in Figure 17 and summarized in Table 11.

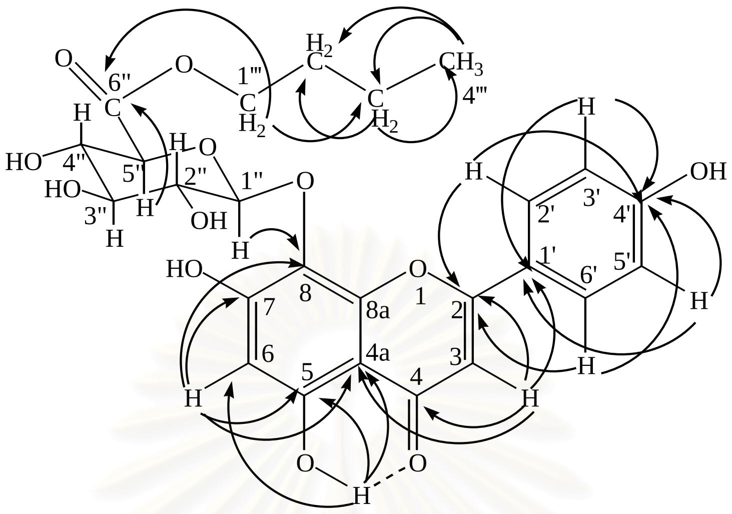


Figure 7 The ^{1}H-^{13}C long-range correlations in the HMBC spectrum of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

Compound PL-3 showed similar spectral data to isoscutellarein 8-*O*-*β*-D-glucuronopyranoside (Billeter *et al.*, 1991). However, the difference was the butyl ester group presented in the sugar moiety. The characteristic aliphatic band also appeared at 2925 cm^{-1} in the IR spectrum. According to these data, compound PL-3 was identified as isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester.

Table 11 The ^{1}H, ^{13}C NMR and HMBC spectral data of PL-3 in DMSO-d_6

Position	PL-3			
	δ_C (ppm)	δ_H (ppm), mult. (*J* in Hz)	HMBC correlations	
			$^nJ_{HC}$ = 8 Hz	$^nJ_{HC}$ = 4 Hz
Aglycone				
2	163.5	-	-	-
3	102.2	6.81, s	C-2, C-4a	C-2, C-4, C-4a, C-1′
4	181.5	-	-	-
4a	103.1	-	-	-
5	157.2	12.78, OH, s	C-4a, C-5, C-6	C-4a, C-5
6	98.9	6.24, s	C-4a, C-5, C-7, C-8	C-4a, C-5, C-7, C-8
7	157.1	-	-	-
8	125.0	-	-	-
8a	149.1	-	-	-
1′	121.0	-	-	-
2′	128.6	8.00, d (8.6)	C-2, C-4′	C-2
3′	115.7	6.87, d (8.6)	C-1′	C-4′
4′	161.0	-	-	-
5′	115.7	6.87, d (8.6)	C-1′	C-4′
6′	128.6	8.00, d (8.6)	C-2, C-4′	C-2
Sugar				
1″	106.3	4.80, d (7.8)	C-8	C-8
2″	73.8	3.0 – 3.5	-	-
3″	75.1	3.0 – 3.5	-	-
4″	71.4	3.0 – 3.5	-	-
5″	75.6	3.87, d (9.7)	C-6″	C-6″
6″	168.6	-	-	-
1‴	64.3	3.97, 2H, dd (6.5, 6.3)	C-6″, C-3‴	-
2‴	30.0	1.36, 2H, m	-	-
3‴	18.5	1.14, 2H, sextet (7.3)	C-2‴, C-4‴	C-4‴
4‴	13.5	0.73, 3H, t (7.3)	C-2‴, C-3‴	C-2‴, C-3‴

4. Structure elucidation of hypolaetin 8-*O*-β-D-glucuronopyranoside butyl ester (PL-4)

Compound PL-4 was obtained as yellow amorphous powder showing optical rotation $[\alpha]^{25}_{D}$ of + 126.90 (c 0.106, MeOH). The UV spectrum in MeOH (Figure 61) exhibited λ_{max} at 272 and 355 nm. The IR spectrum (Figure 62) displayed characteristic bands at 3071 (hydroxyl group), 2738 (aliphatic), 1739 (ester carbonyl), 1656 (ketone carbonyl), and 1576 cm^{-1} (aromatic ring). The FABMS spectrum (Figure 63), showing the pseudomolecular ion peak at m/z 535 $[M+H]^+$, established the molecular formula of this compound as $C_{25}H_{26}O_{13}$.

The 300 MHz ^{1}H NMR spectrum in DMSO-d_6 of compound PL-4 (Figures 64-65) displayed one methyl proton signal at δ 0.74 ppm ; three methylene proton signals at δ 1.14, 1.35, and 3.95 ppm ; five oxymethine proton signals at δ 3.0-3.5 (3H), 3.85, and 4.81 ppm ; five aromatic and olefenic proton signals at δ 6.26, 6.63, 6.86, 7.38, and 7.56 ppm; and a chelated hydroxyl signal at δ 12.79 ppm. The 75 MHz ^{13}C NMR spectrum in DMSO-d_6 (Figure 66) indicated twenty-five carbons, consistent with the molecular formula. The carbon signals were classified by the DEPT 135 spectrum (Figure 67) as one methyl carbon signal at δ 13.5 ppm ; three methylene carbon signals at δ 18.4, 30.0, and 64.4 ppm ; five methine carbon signals at δ 71.4, 73.8, 75.3, 75.7 and 106.0 ppm ; five aromatic and olefinic carbon signals at δ 99.0, 102.4, 113.7, 115.8, and 119.6 ppm ; and eleven quaternary carbon signals at δ 103.5, 121.5, 125.0, 145.7, 149.5, 150.0, 156.9, 157.4, 164.1, 168.8, and 181.9 ppm.

Analyses of the HMQC spectrum (Figures 68-69) facilitated in the assignments of protons and their respective carbons, as shown in Table 12. The ^{1}H-^{1}H COSY spectrum (Figure 70) exhibited the proton connectivities in the aglycone and sugar moiety as follows : H-2′ / H-6′ / H-5′ ; H-1″ / H-2″ ; H-4″ / H-5″ ; H-1‴ / H-2‴ / H-3‴ / H-4‴ (Figure 8).

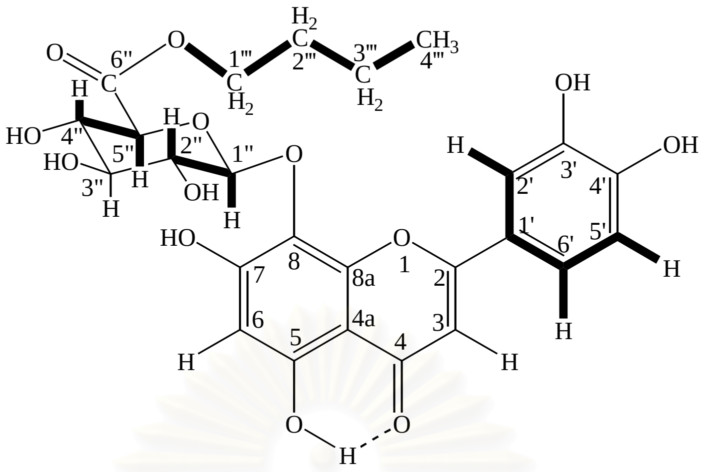


Figure 8 The ^{1}H-^{1}H correlations (bold line) in the ^{1}H-^{1}H COSY spectrum of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

The proton and carbon resonances in the ^{1}H and ^{13}C NMR spectra of compound PL-4 and hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (Compound PL-2) showed both compounds as having identical aglycone structure but differing in the sugar moiety part. From the presence of butyl ester group and the correlation of H-1‴ (δ 3.95 ppm) to C-6″ (δ 168.8 ppm), The proposed structure of compound PL-4 was hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester.

The complete NMR assignments of compound PL-4 were confirmed by the HMBC ($^nJ_{HC}$ = 8 and 4 Hz) spectra (Figures 71-75). The ^{1}H-^{13}C long-range correlations are shown in Figure 9 and summarized in Table 12.

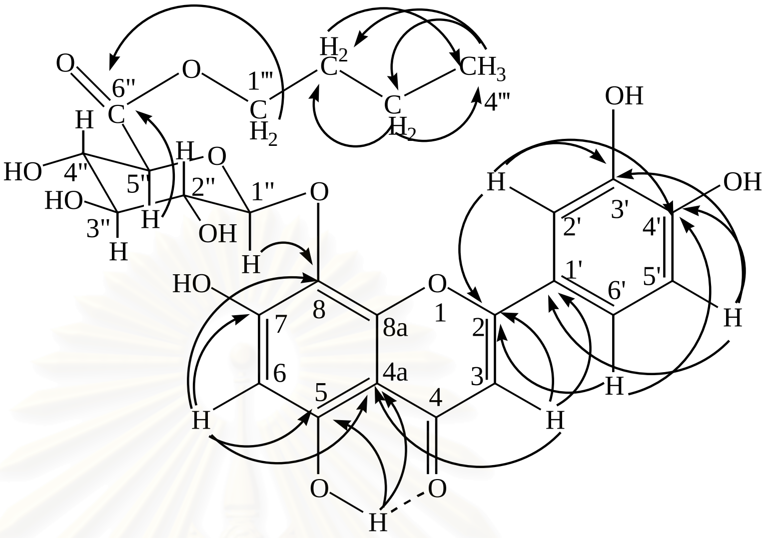


Figure 9 The ^{1}H-^{13}C long-range correlations in the HMBC spectrum of hypolaetin 8-*O*-β-D-glucuronopyranoside butyl ester (PL-4)

Table 12 The ^{1}H, ^{13}C NMR and HMBC spectral data of PL-4 in DMSO-d_6

Position	PL-4			
	δ_C (ppm)	δ_H (ppm), mult. (*J* in Hz)	HMBC correlations	
			$^nJ_{HC}$ = 8 Hz	$^nJ_{HC}$ = 4 Hz
Aglycone				
2	164.1	-	-	-
3	102.4	6.63, s	C-2, C-4a	C-1´, C-4a
4	181.9	-	-	-
4a	103.5	-	-	-
5	157.4	12.79, OH, s	C-4a, C-5, C-6	C-4a
6	99.0	6.26, s	C-4a, C-5, C-6, C-7	C-4a, C-5, C-7, C-8
7	156.9	-	-	-
8	125.0	-	-	-
8a	150.0	-	-	-
1′	121.5	-	-	-
2′	113.7	7.38, d (1.9)	C-2, C-4′, C-3′	C-2, C-3′, C-4′
3′	145.7	-	-	-
4′	149.5	-	-	-
5′	115.8	6.86, d (8.4)	C-1′, C-3′	C-1′, C-3′, C-4′
6′	119.6	7.56, dd (8.4, 1.9)	C-4′	C-2
Sugar				
1″	106.0	4.81, d (7.8)	C-8	C-8
2″	73.8	3.0 – 3.5	-	-
3″	75.3	3.0 – 3.5	-	-
4″	71.4	3.0 – 3.5	-	-
5″	75.7	3.85, d (9.7)	C-6″	C-6″
6″	168.8	-	-	-
1‴	64.4	3.95, 2H, dd (6.2, 5.6)	-	-
2‴	30.0	1.35, 2H, m	-	C-4‴
3‴	18.4	1.14, 2H, sextet (7.3)	C-2‴	C-4‴
4‴	13.5	0.74, 3H, t (7.3)	C-2‴, C-3‴	C-2‴, C-3‴

CHAPTER V

CONCLUSION

In this investigation, the butanol extract from the leaves of *Ploiarium alternifolium* was mainly isolated by high speed countercurrent chromatography (HSCCC). This technique led to the isolation of four yellowish polar components. These compounds were determined as the flavone glycosides, isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester, hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester, isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester, and hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester. Their yields were 3.1×10^{-3}, 2.0×10^{-3}, 6.7×10^{-4}, and 2.4×10^{-3} % w/w based on dried weight of the leaves, respectively. Both isoscutellarein and hypolaetin were the known aglycones isolated from many plants (Harborne, 1994). The glucuronic acid and its ester derivatives were found in some glycosides. The previous phytochemical study in *Helicteres angustifolia* (Sterculiaceae) reporetd that the glucuronide methyl ester was linked with takakin to be the compound, takakin 8-*O*-*β*-D-glucuronopyranoside metyl ester(Chen, Lee and Chen,1994). However, the linkage between isoscutellarein or hypolaetin with these sugar parts have never been reported. The investigation of of *P. alternifolium* showed the presence of xanthones, anthraquinones, and triterpenoids in the barks and branches (Bennett *et al.*, 1990; 1991; 1992). But chemical constituents of its leaves have never been reported. Therefore, this is the first report of flavone glycosides from *P. alternifolium* leaves.

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APPENDIX

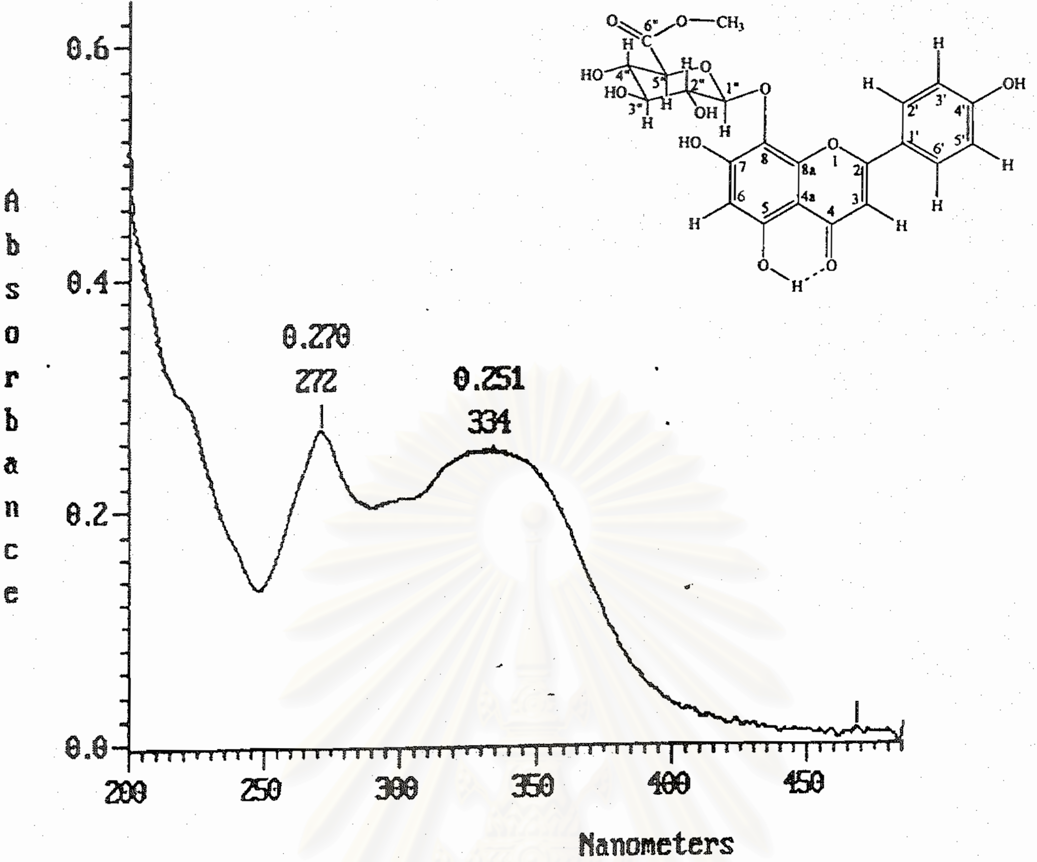


Figure 10 The UV spectrum (in MeOH) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

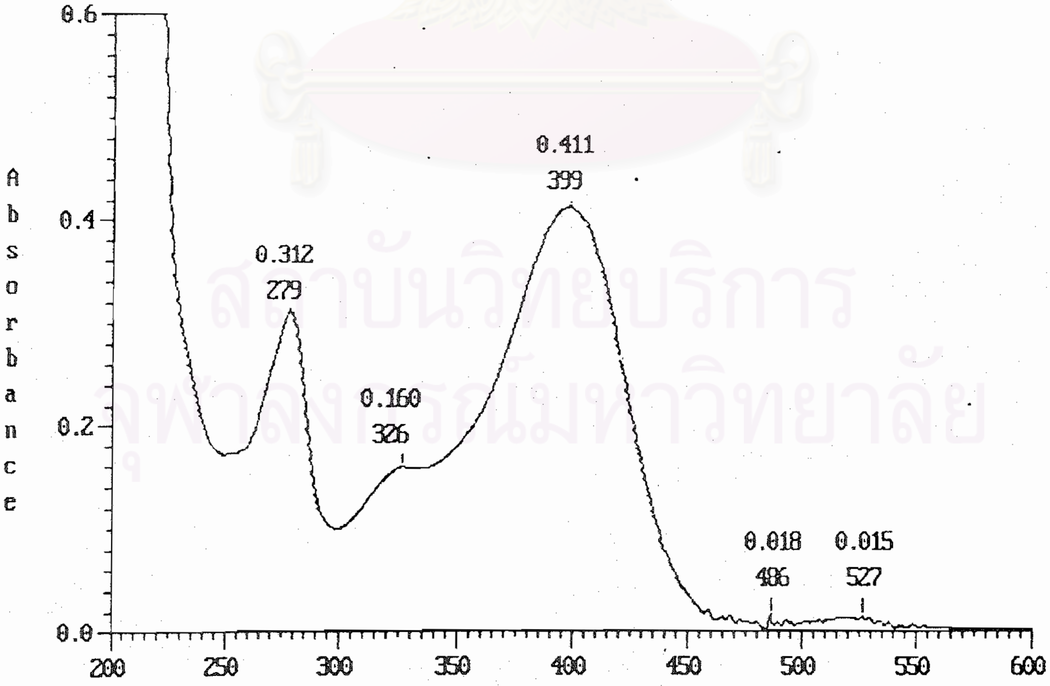


Figure 11 The UV spectrum (in MeOH + NaOH) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

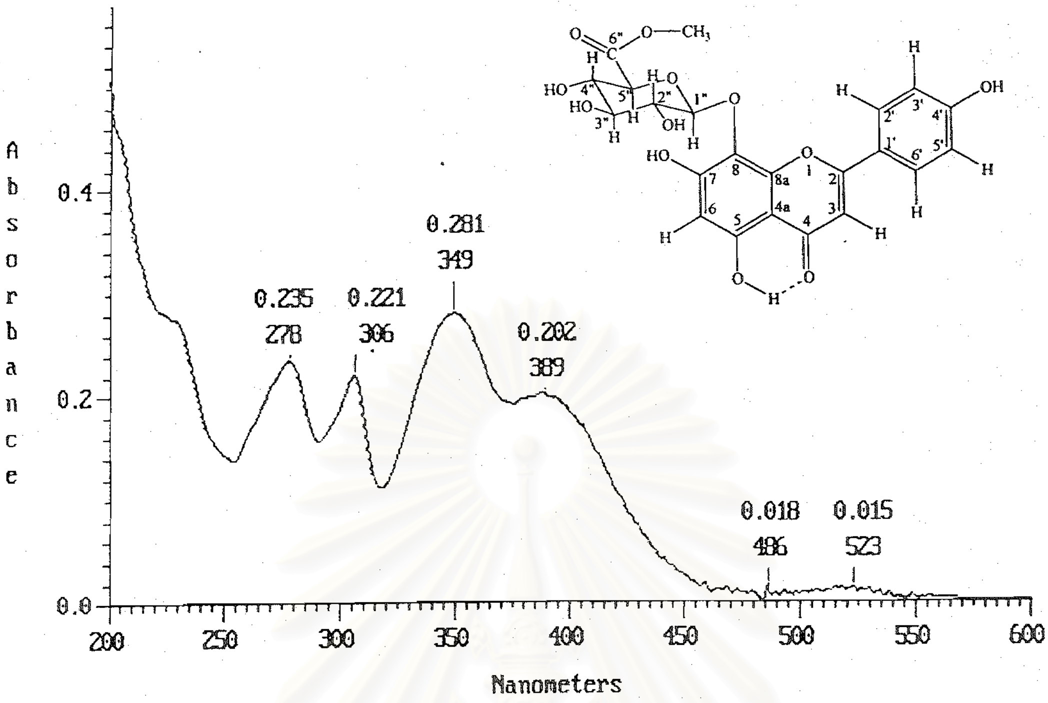


Figure 12 The UV spectrum (in MeOH + $AlCl_3$) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

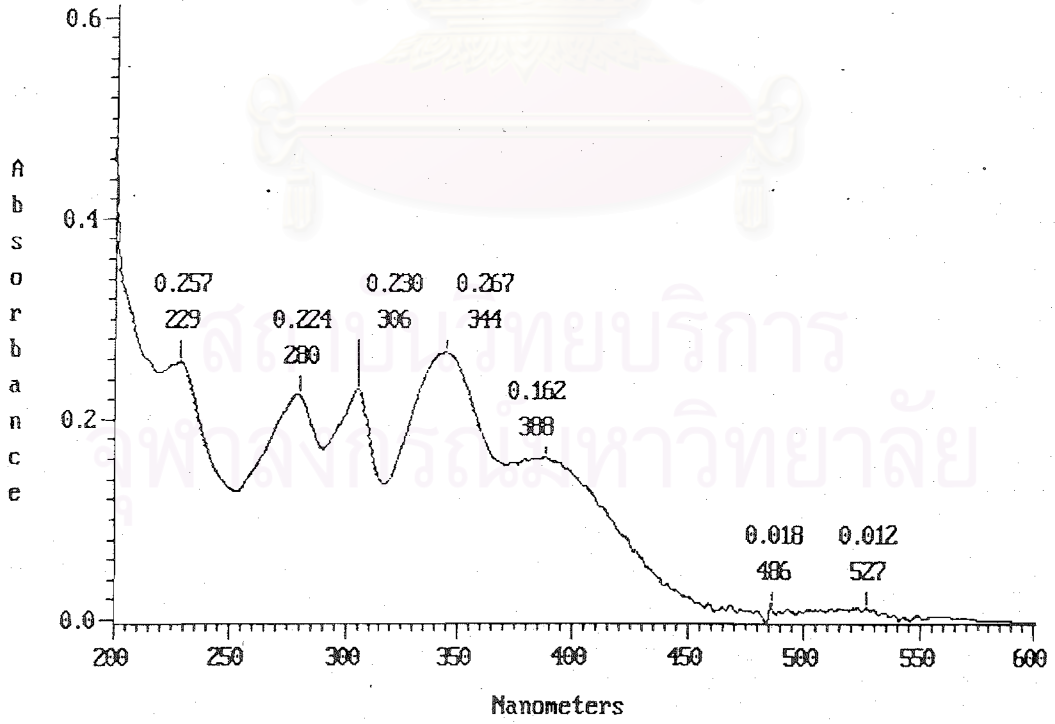


Figure 13 The UV spectrum (in MeOH + $AlCl_3$ + HCl) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

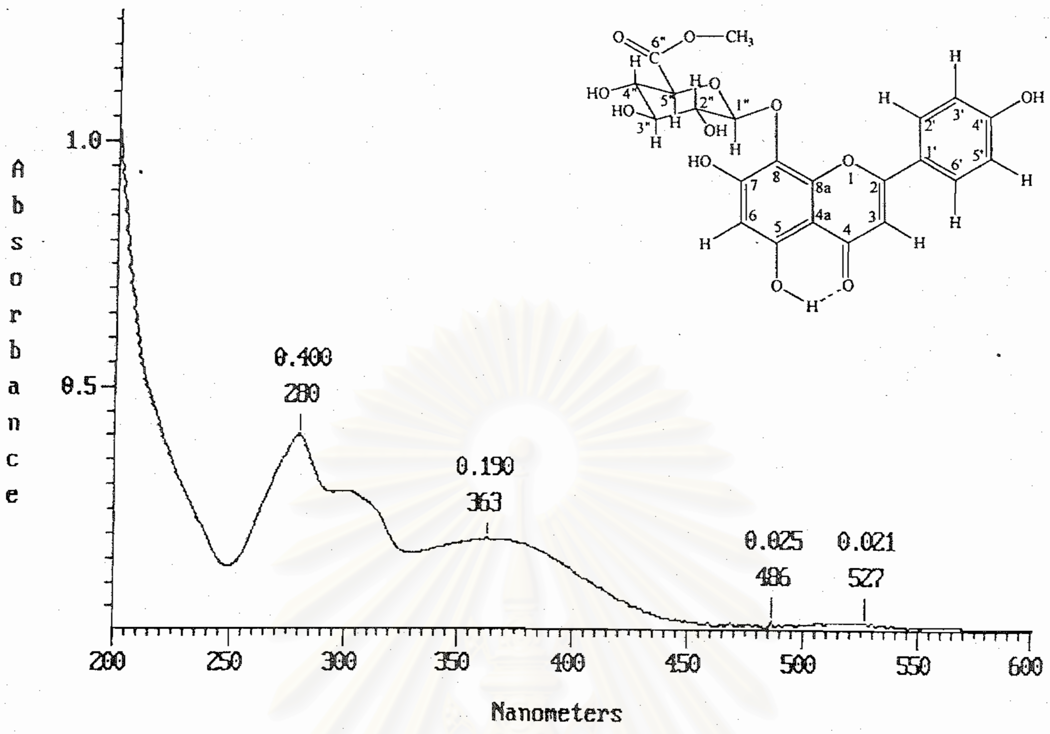


Figure 14 The UV spectrum (in MeOH + NaOAc) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

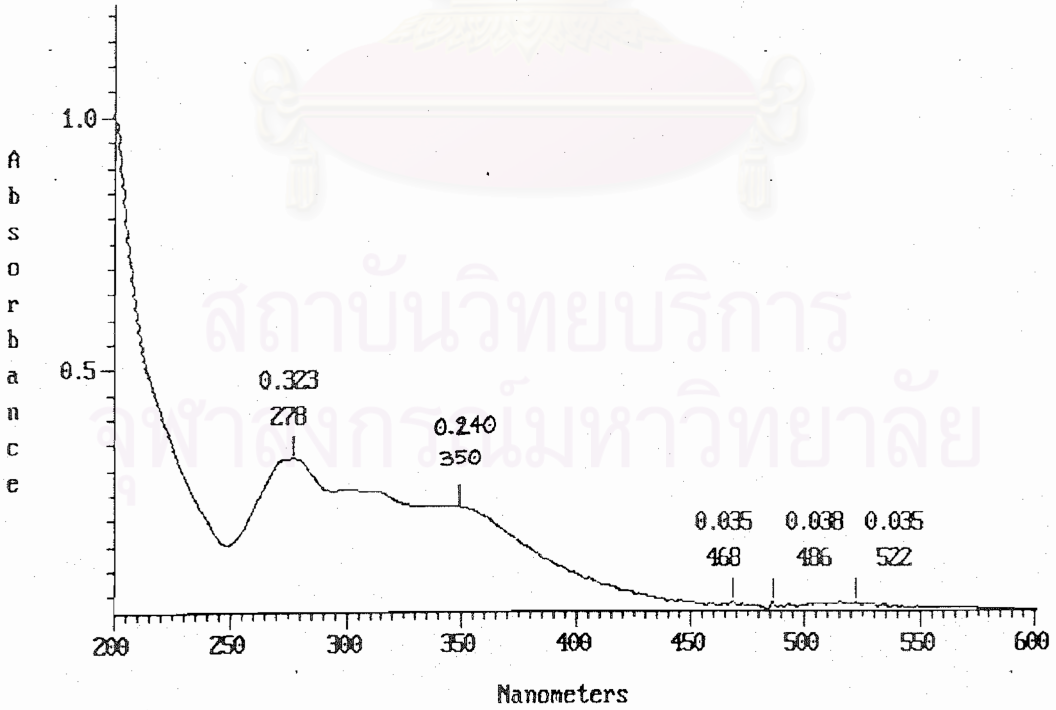


Figure 15 The UV spectrum (in MeOH + NaOAc + H_3BO_3) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

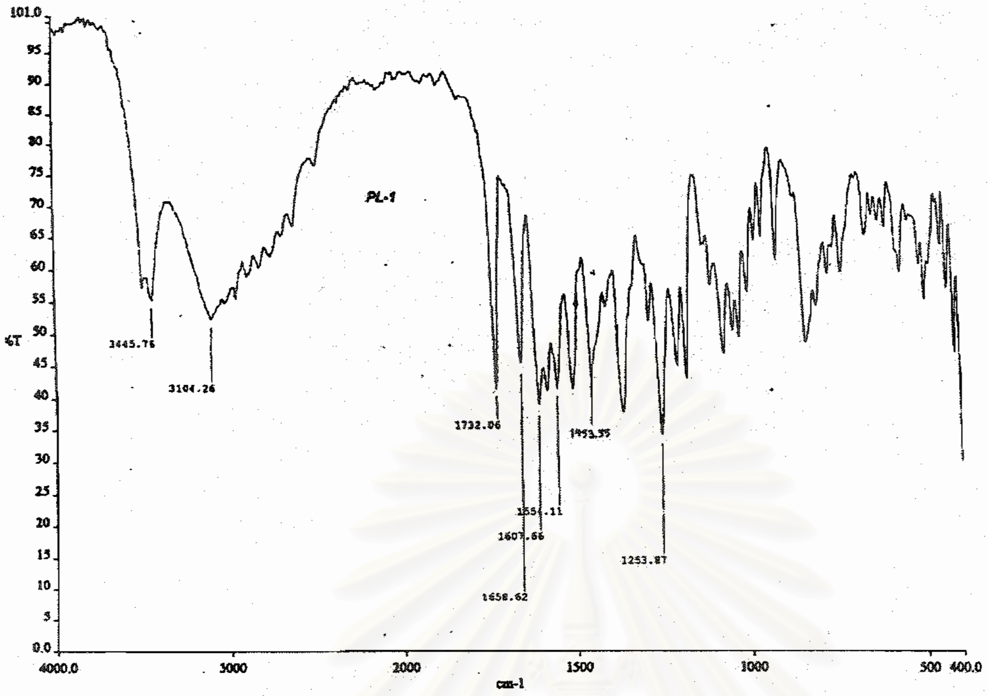


Figure 16 The IR spectrum (in KBr disc) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

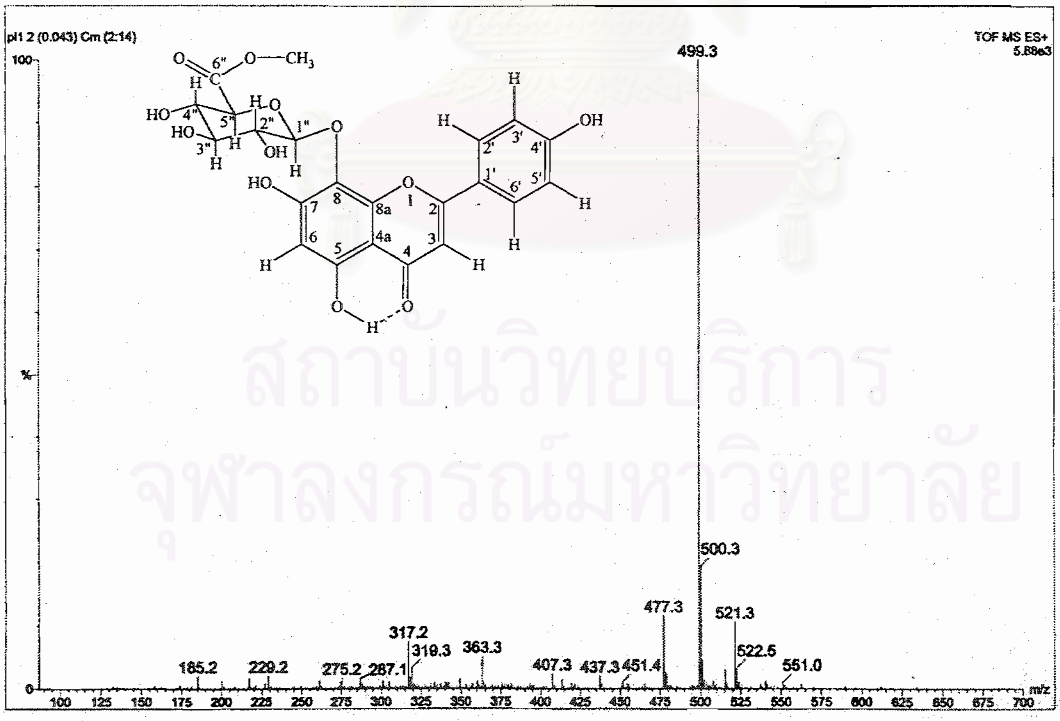


Figure 17 The ESI-TOF mass spectrum of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

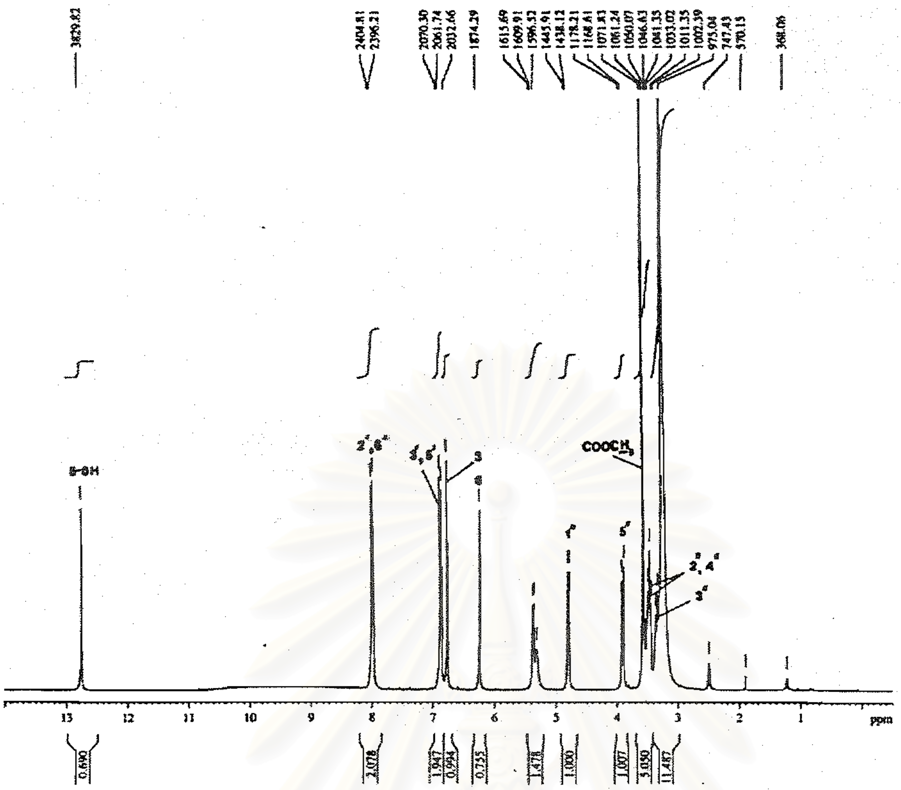


Figure 18 The 300 MHz ^{1}H NMR spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

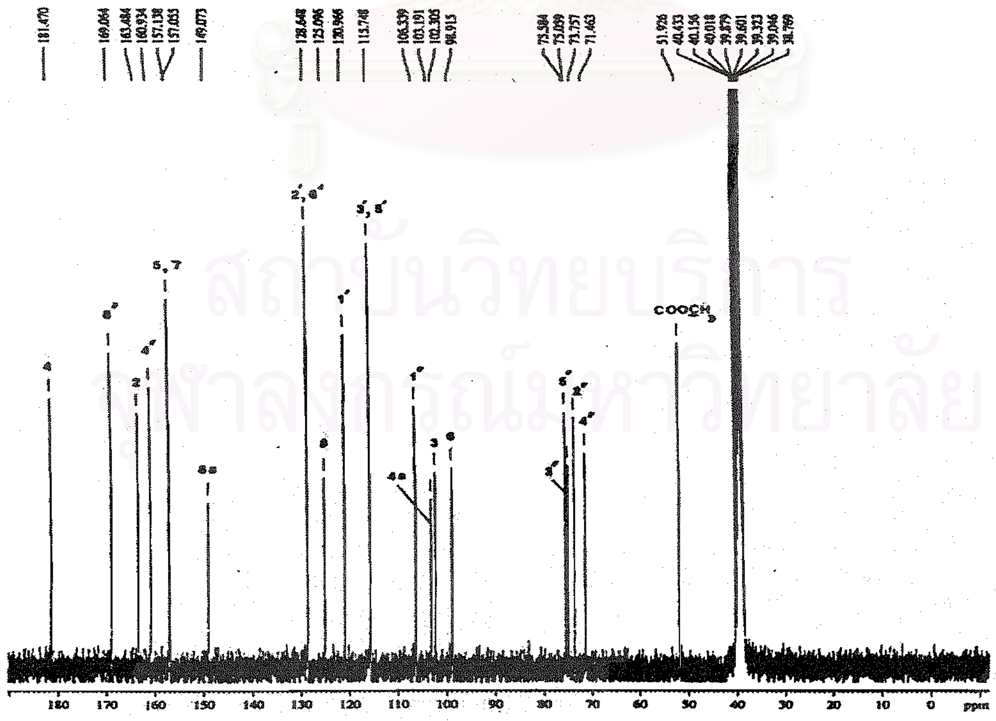


Figure 19 The 75 MHz ^{13}C NMR spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

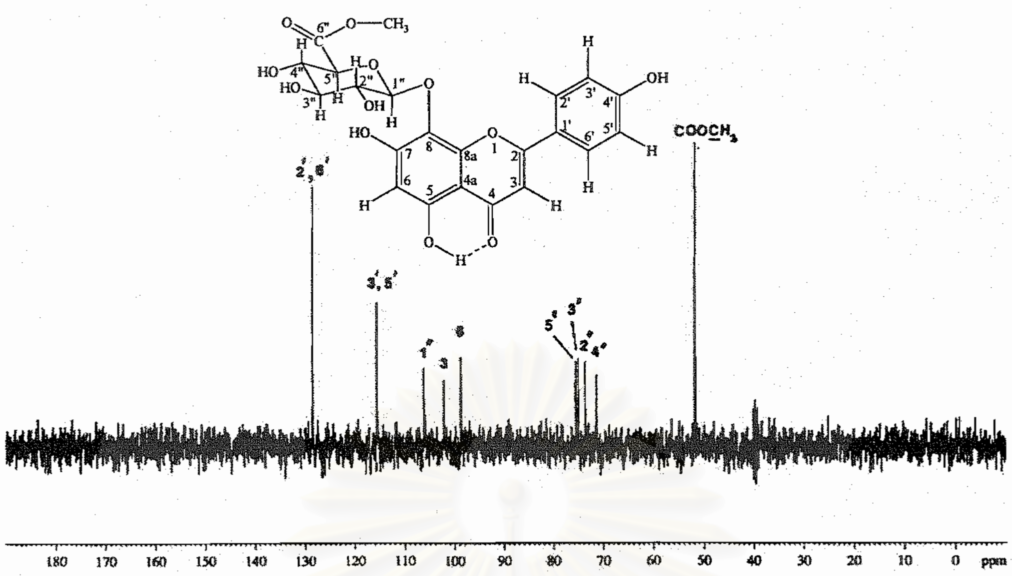


Figure 20 The 75 MHz DEPT 135 NMR spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

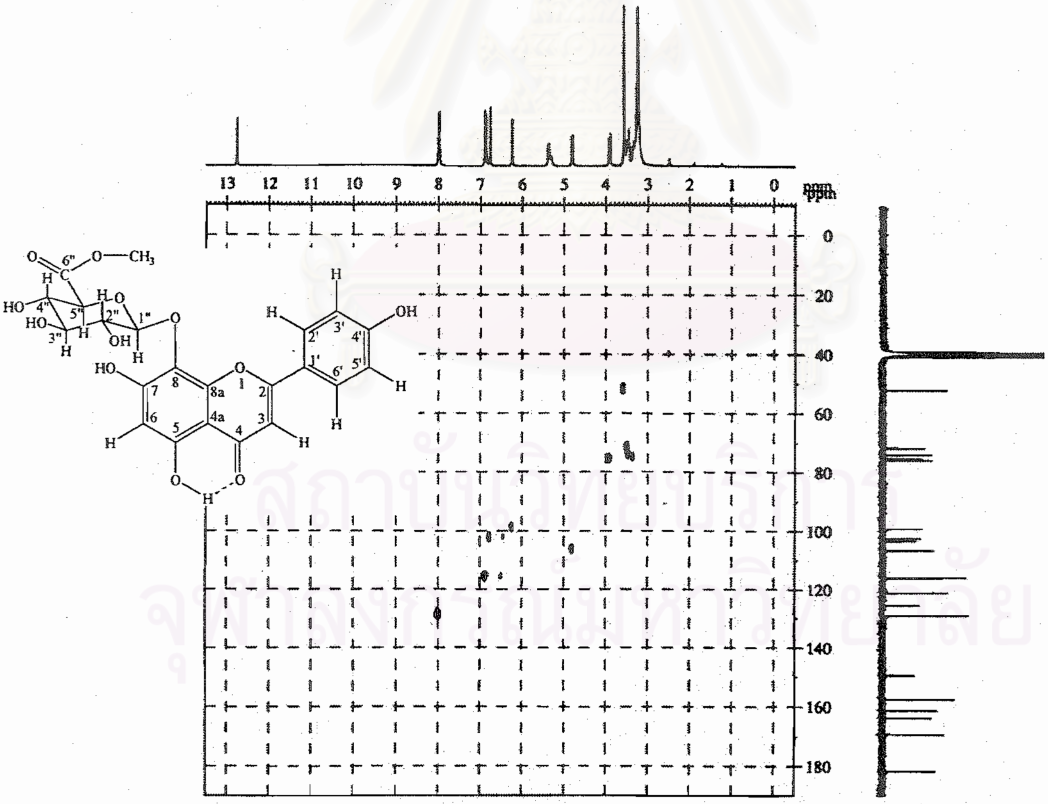


Figure 21 The 300 MHz HMQC spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

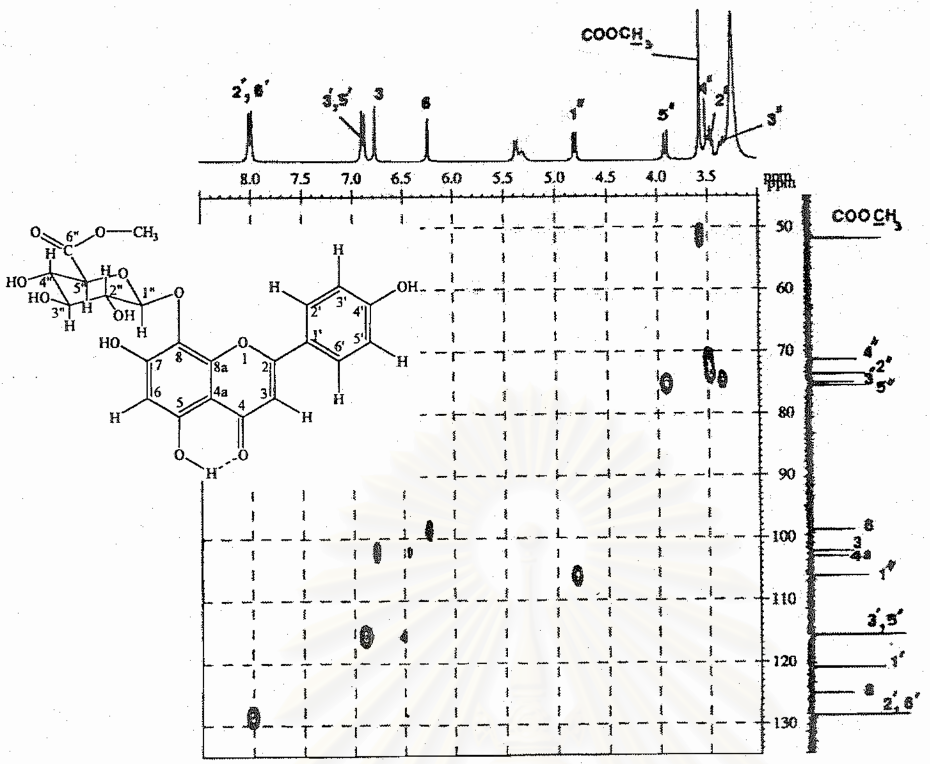


Figure 22 The HMQC spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-β-D-glucuronopyranoside methyl ester (PL-1) (expanded from δ_H 3.00 – 8.50 ppm and δ_C 45.0 – 135.0 ppm

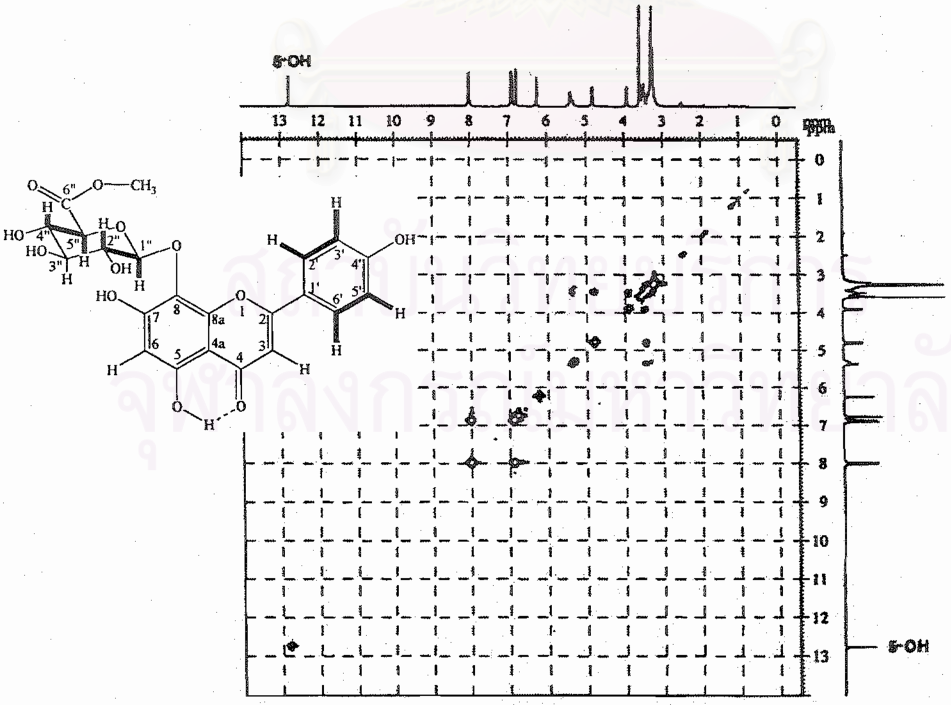


Figure 23 The 300 MHz ^{1}H-^{1}H COSY spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-β-D-glucuronopyranoside methyl ester (PL-1)

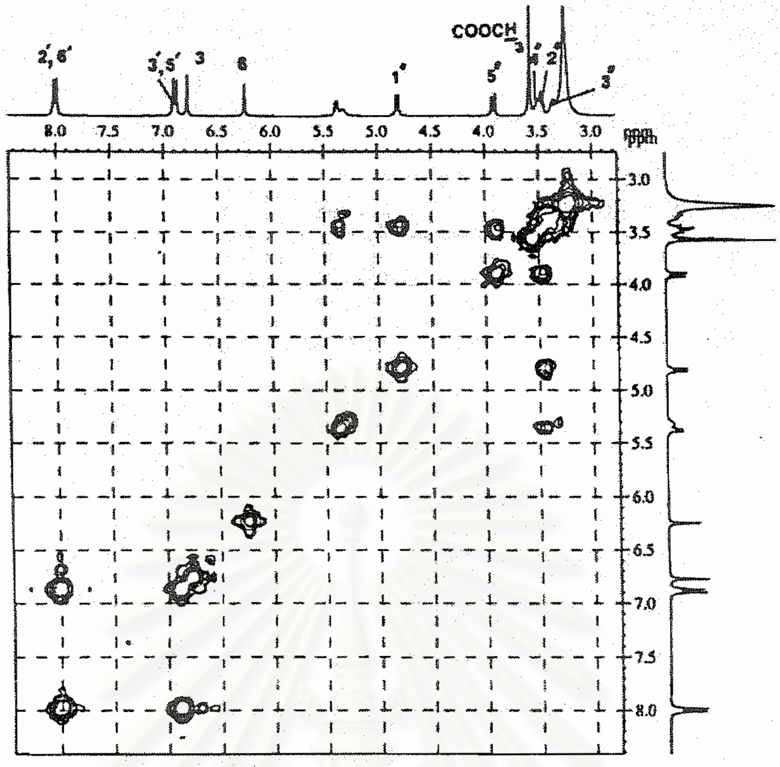


Figure 24 The 300 MHz ^1H-^1H COSY spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1) (expanded from δ_H 2.80-8.50 ppm)

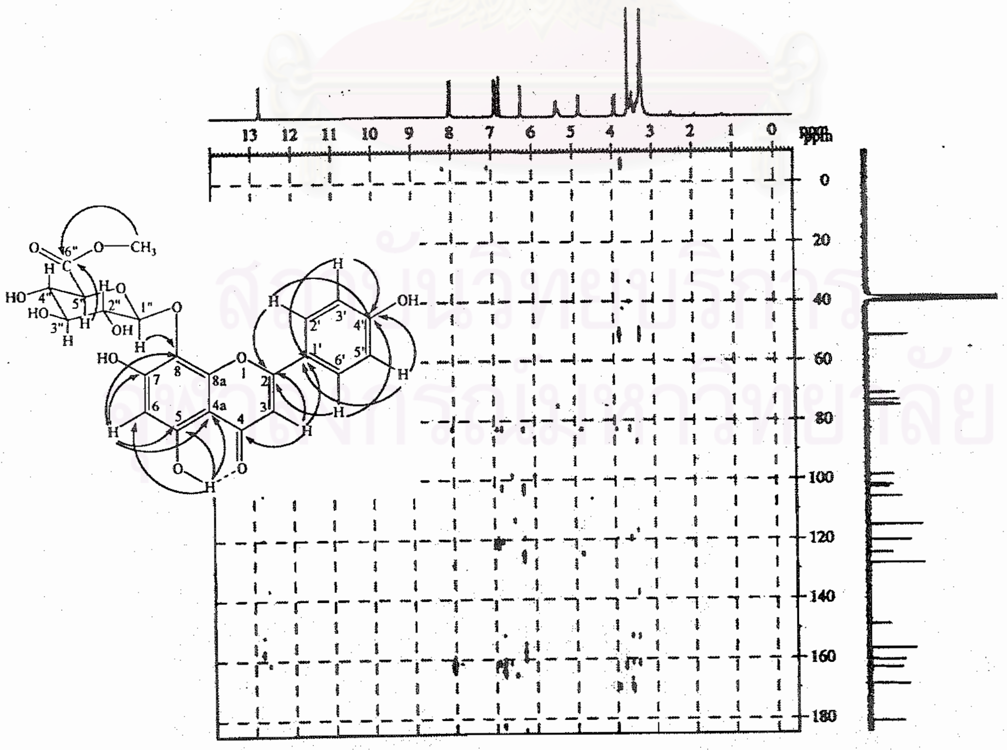


Figure 25 The HMBC spectrum ($^nJ_{HC}$ = 8 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

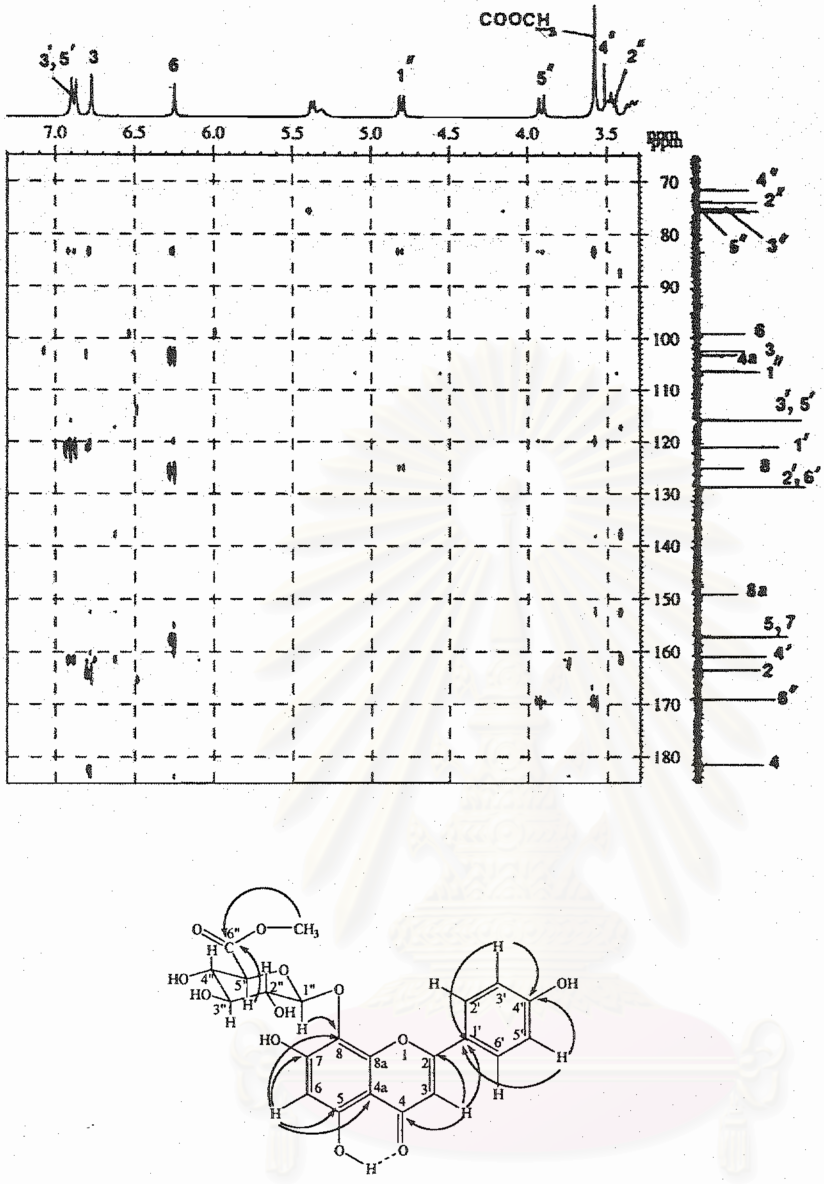


Figure 26 The HMBC spectrum (${}^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1) (expanded from δ_H 3.20 – 7.30 ppm and δ_C 65.0 – 185.0 ppm

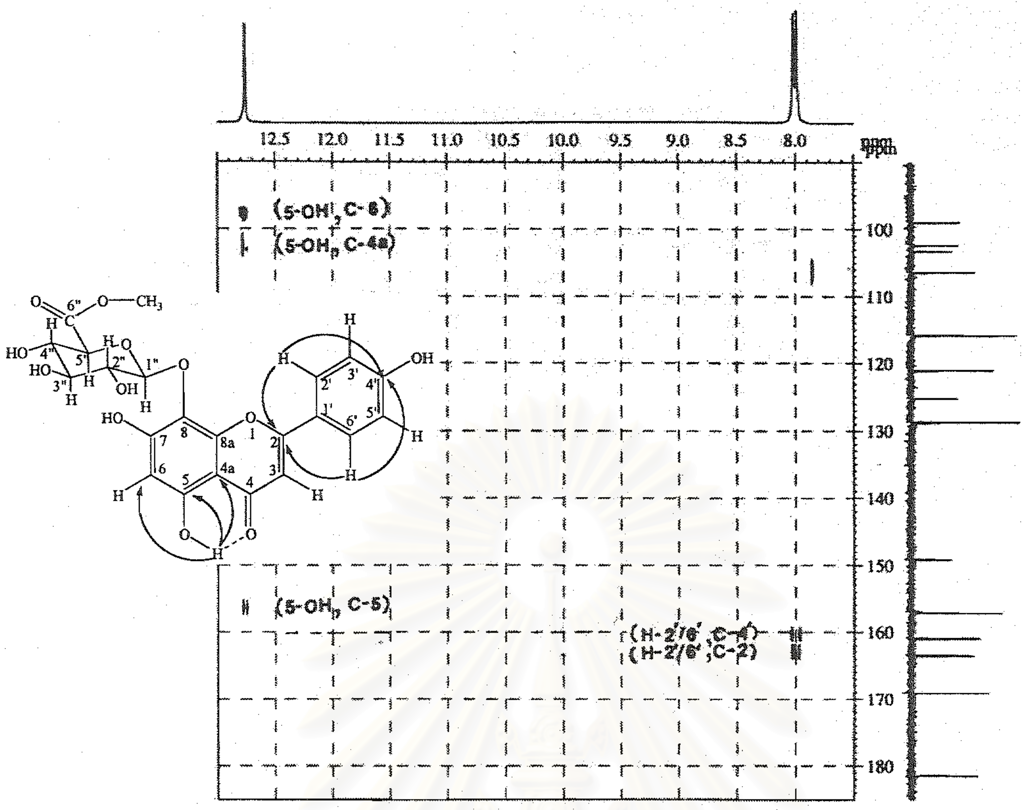


Figure 27 The HMBC spectrum ($^nJ_{HC}$ = 8 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1) (expanded from δ_H 7.50 – 13.00 ppm and δ_C 90.0 – 185.0 ppm

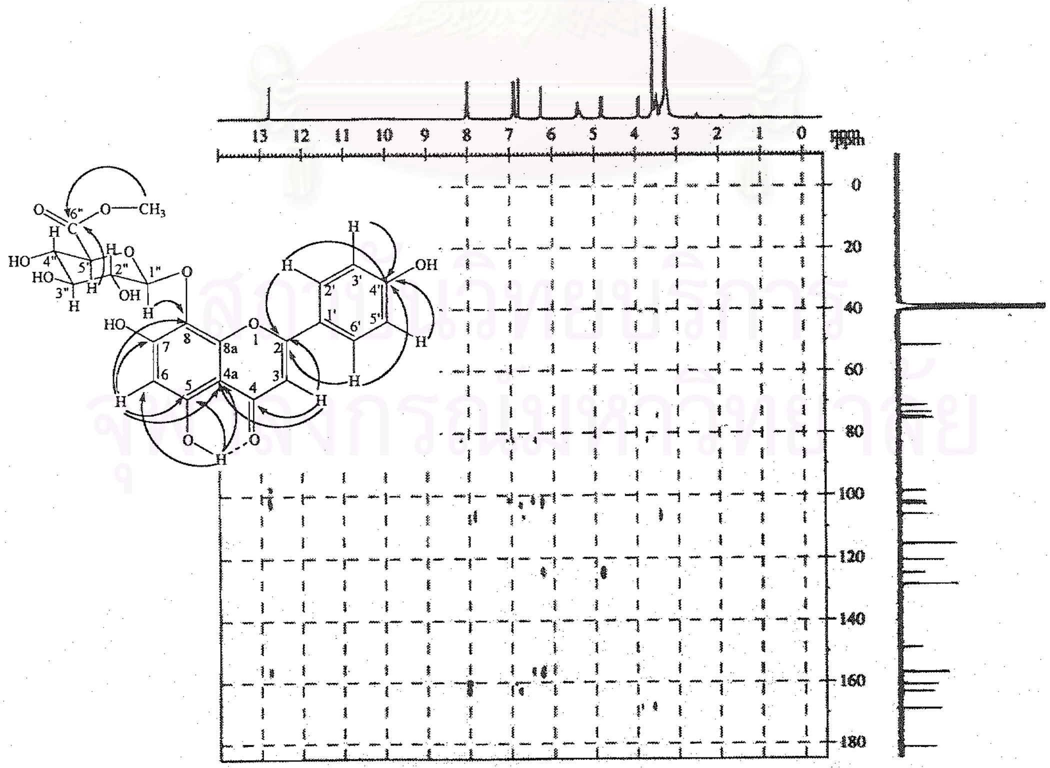


Figure 28 The HMBC spectrum ($^nJ_{HC}$ = 4 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-1)

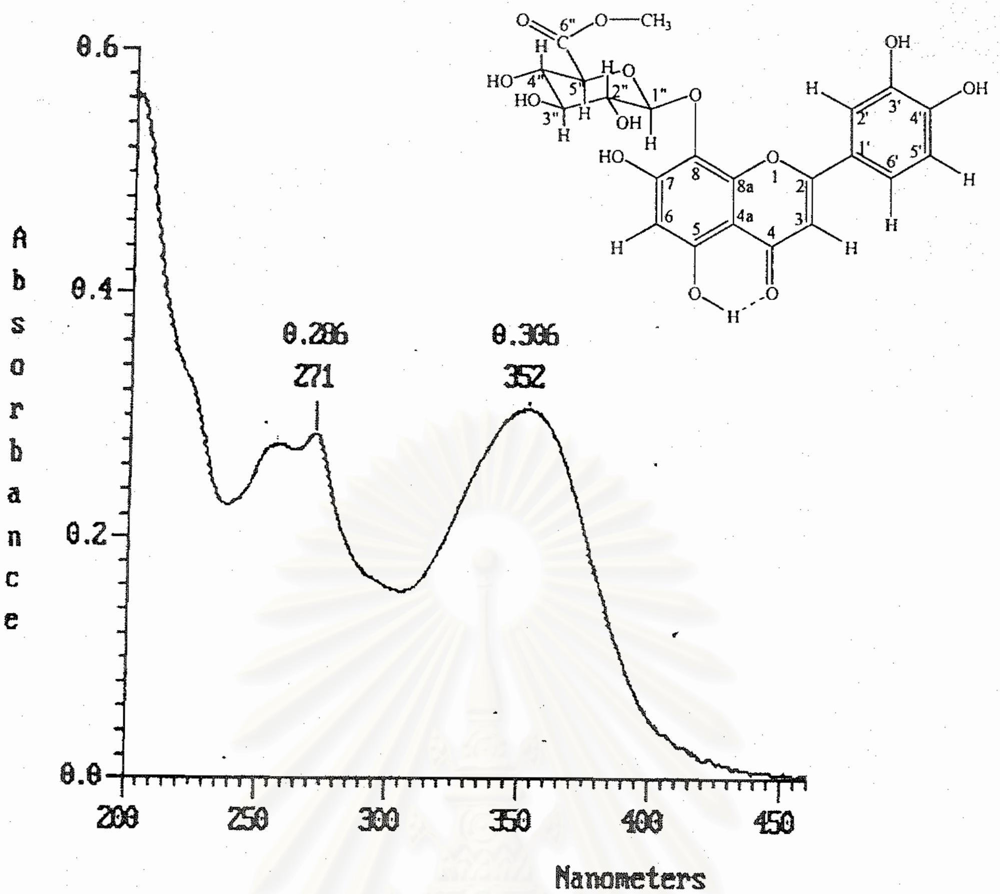


Figure 29 The UV spectrum (in MeOH) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

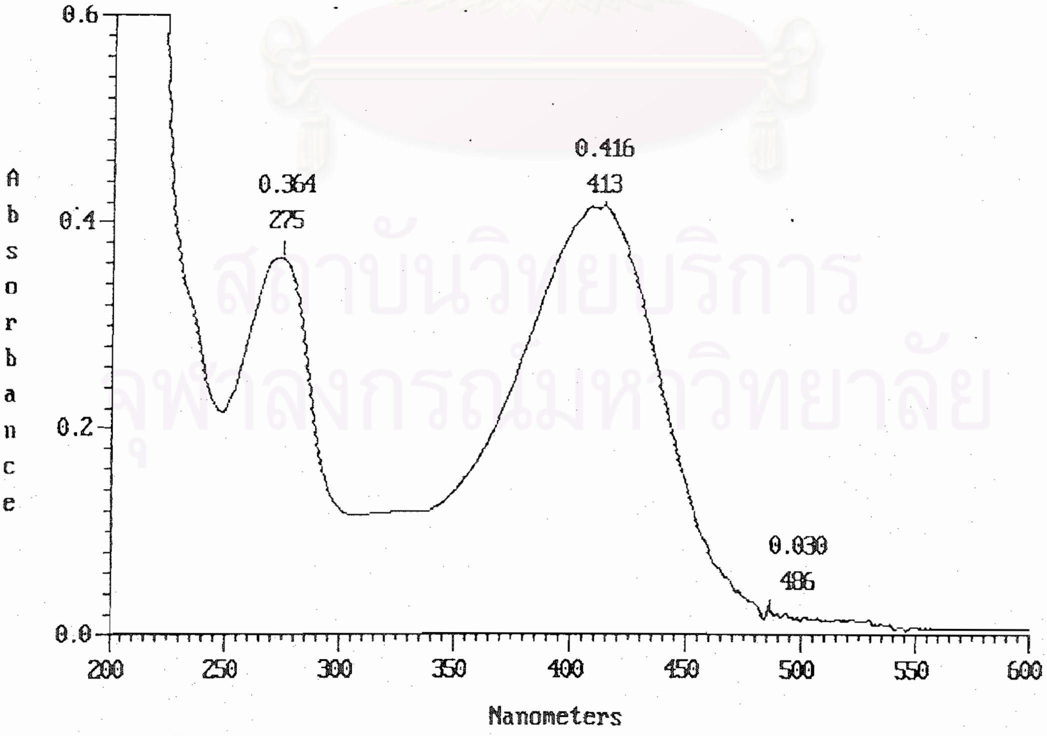


Figure 30 The UV spectrum (in MeOH + NaOH) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

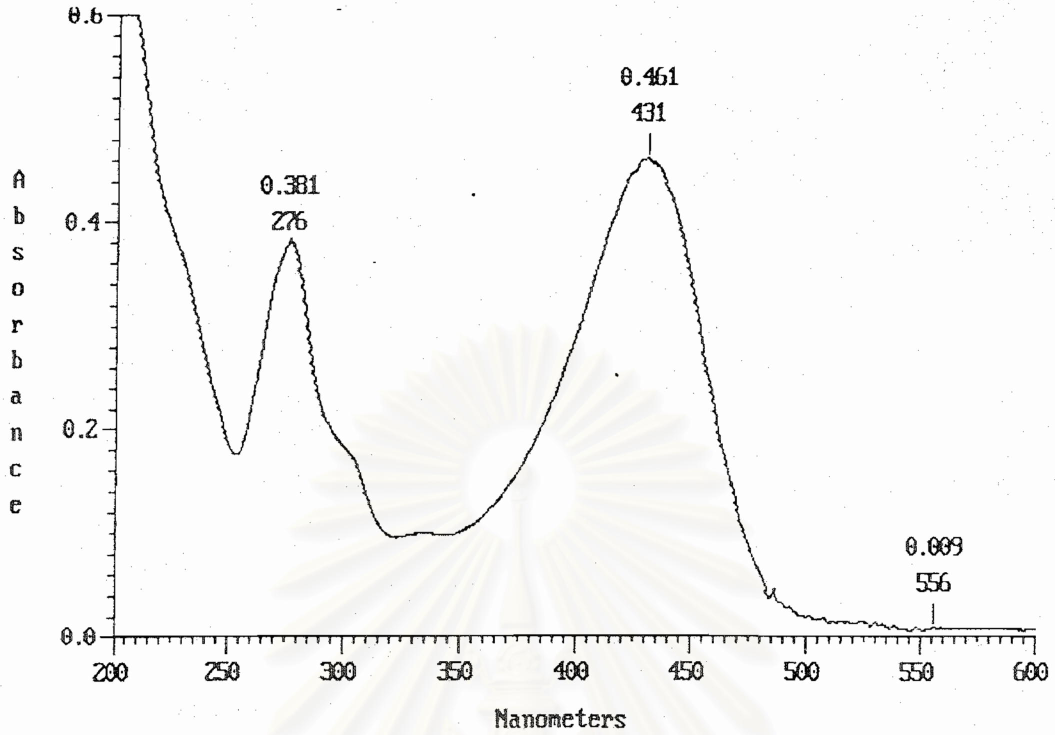


Figure 31 The UV spectrum (in MeOH +$AlCl_3$) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

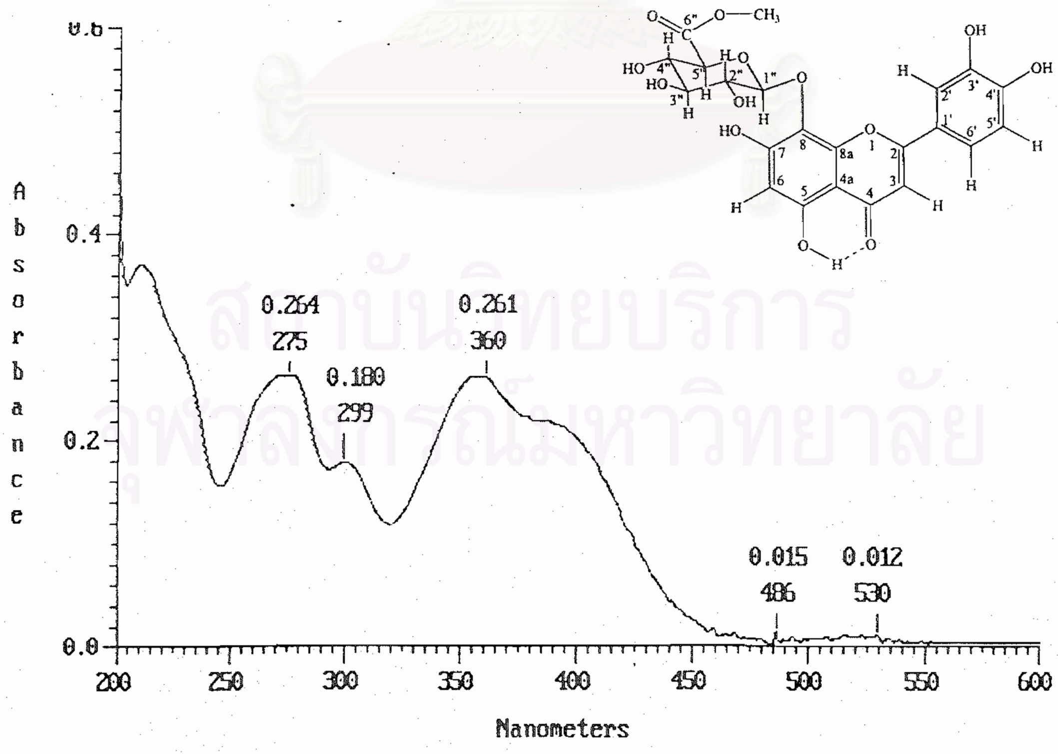


Figure 32 The UV spectrum (in MeOH + $AlCl_3$ + HCl) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

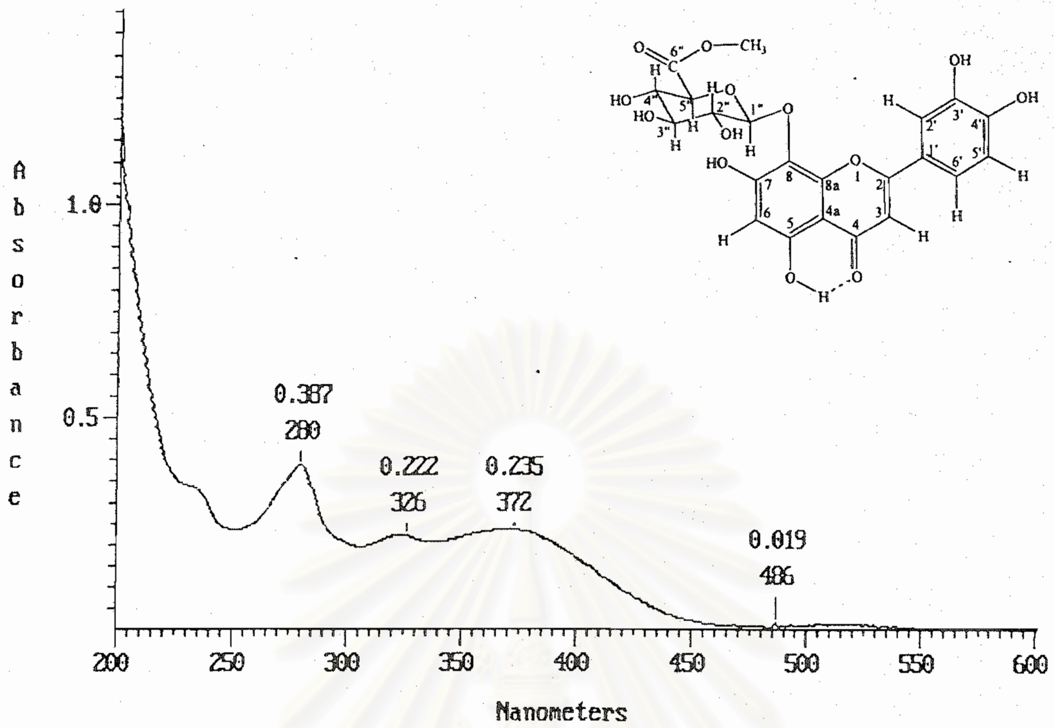


Figure 33 The UV spectrum (in MeOH + NaOAc) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

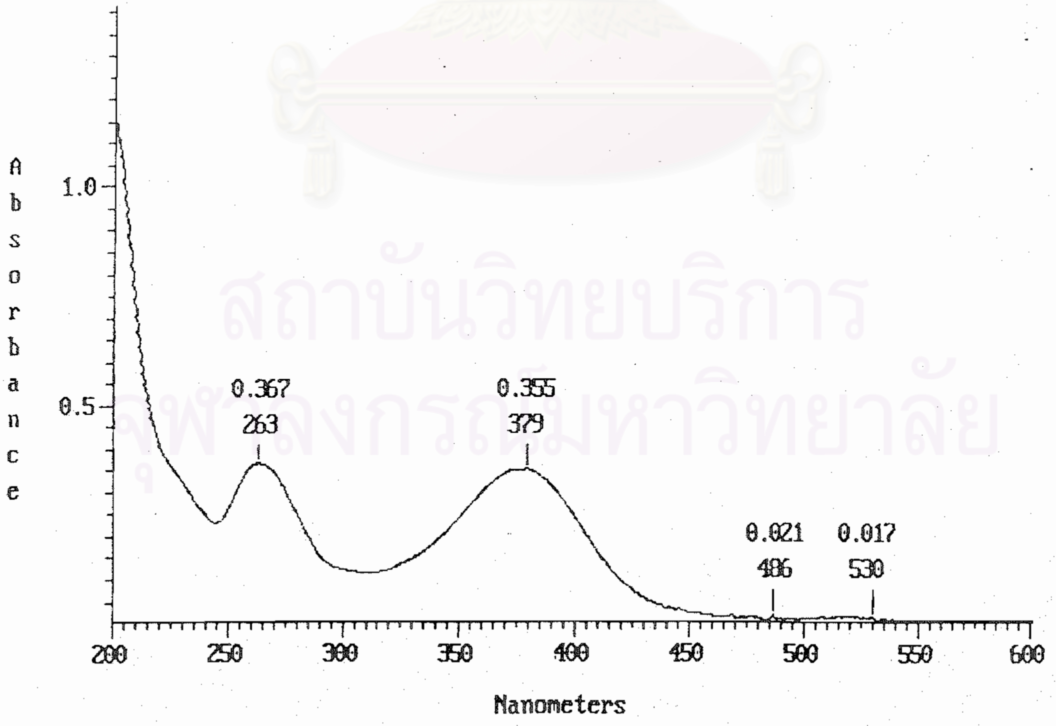


Figure 34 The UV spectrum (in MeOH + NaOAc + H_3BO_3) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

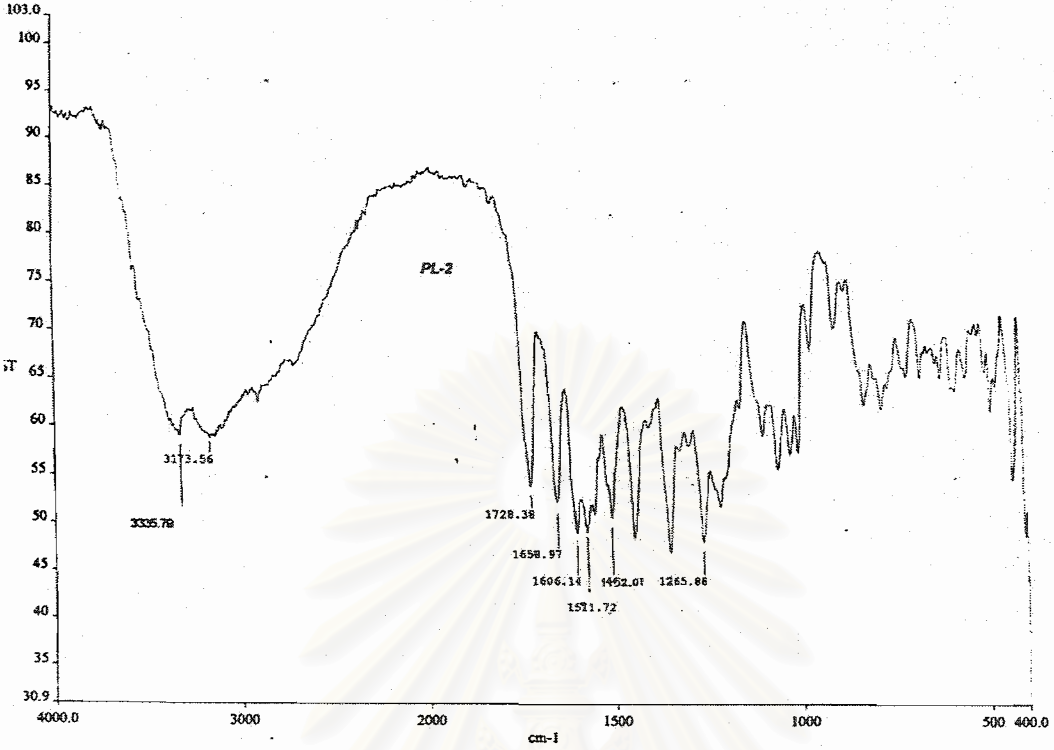


Figure 35 The IR spectrum (in KBr disc) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

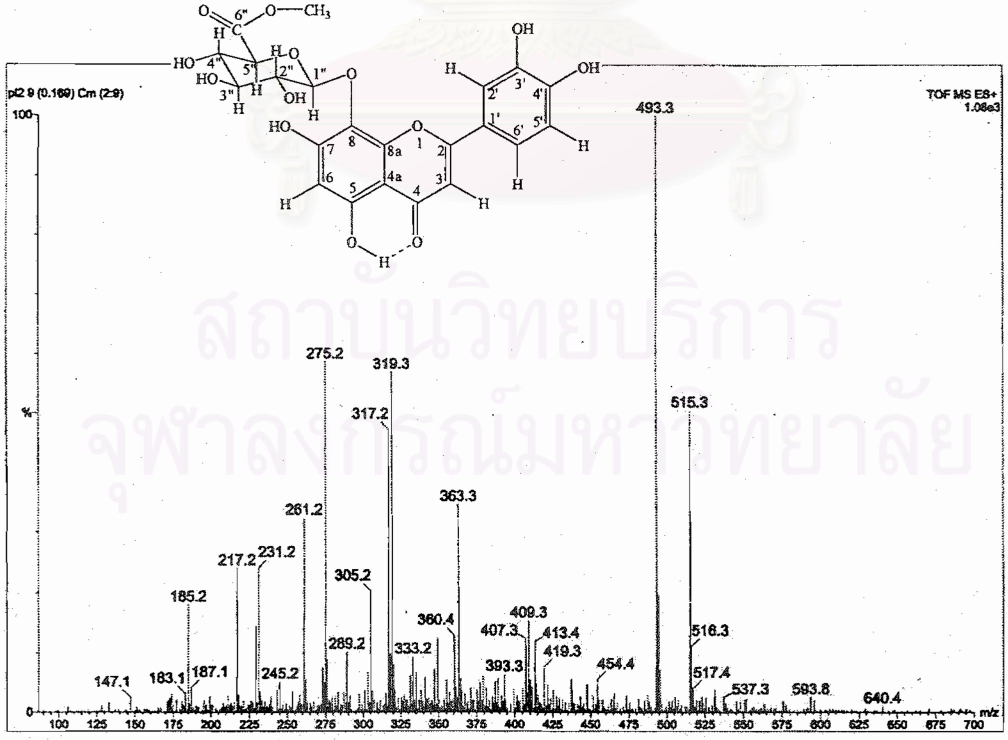


Figure 36 The ESI-TOF mass spectrum of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

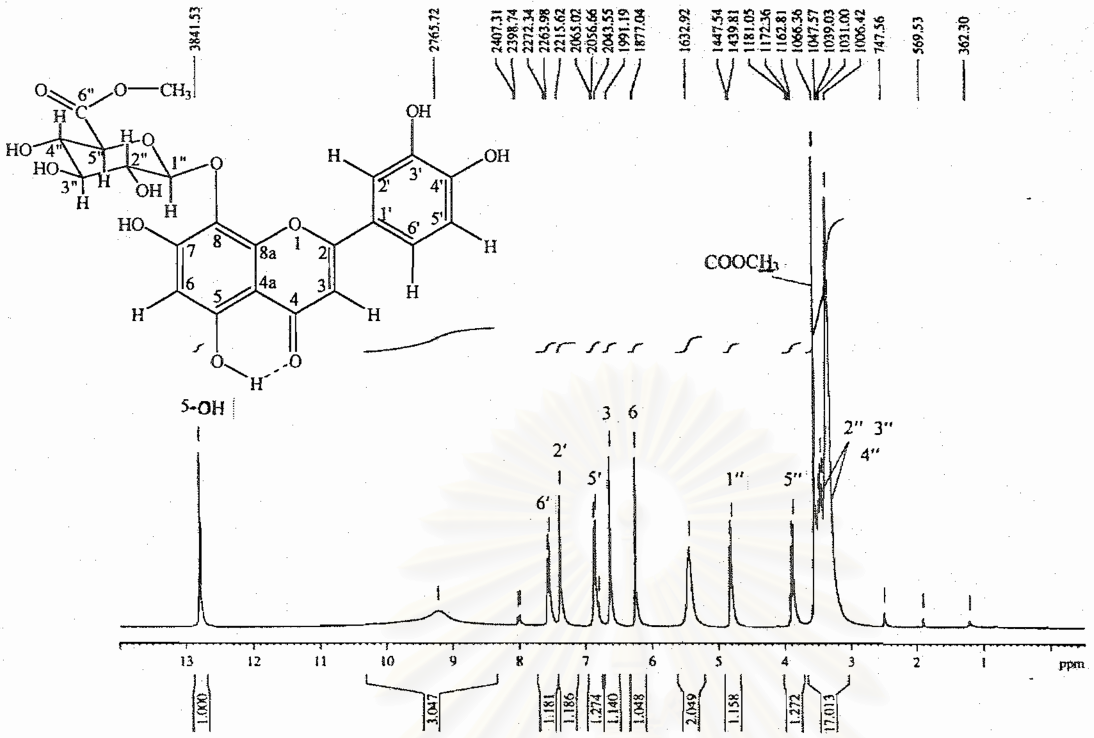


Figure 37 The 300 MHz ^{1}H NMR spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

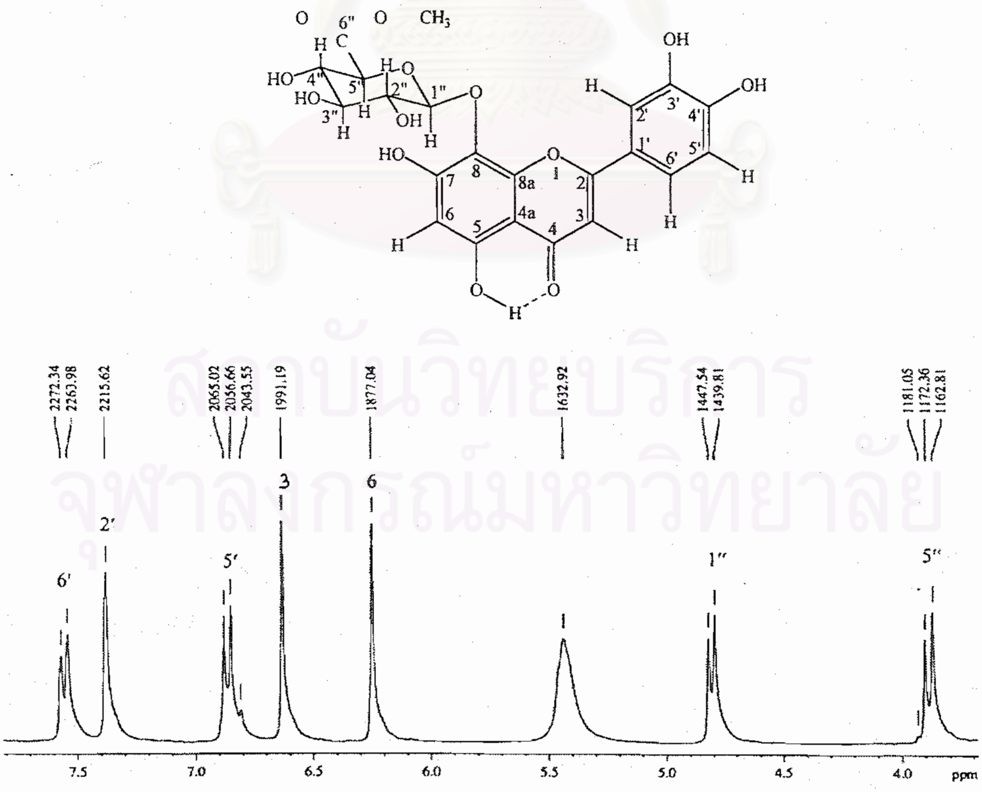


Figure 38 The 300 MHz ^{1}H NMR spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2) (expanded from δ_H 3.68-7.81 ppm)

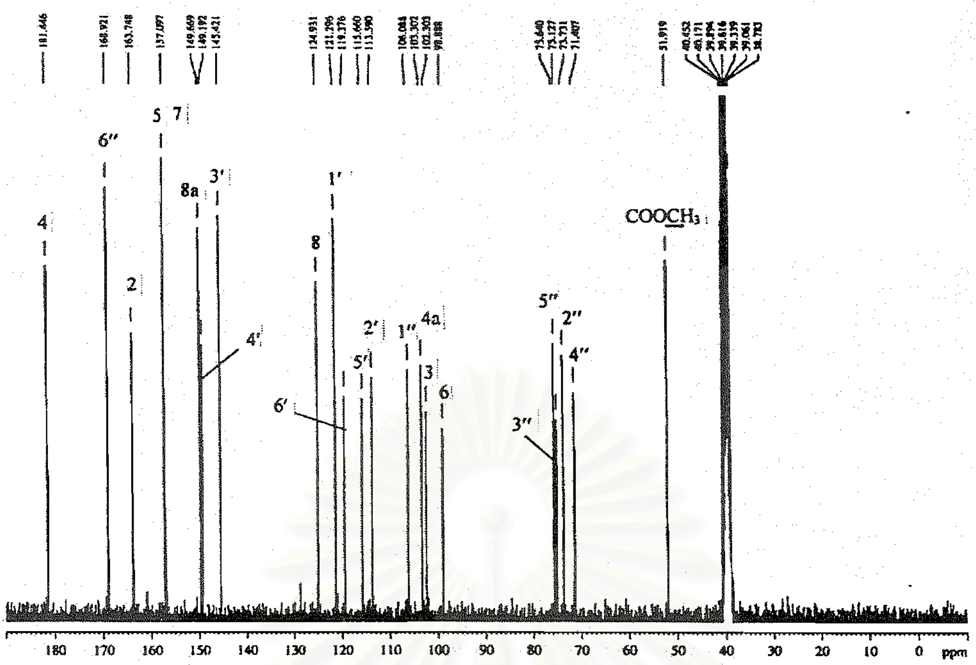


Figure 39 The 75 MHz ^{13}C NMR spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

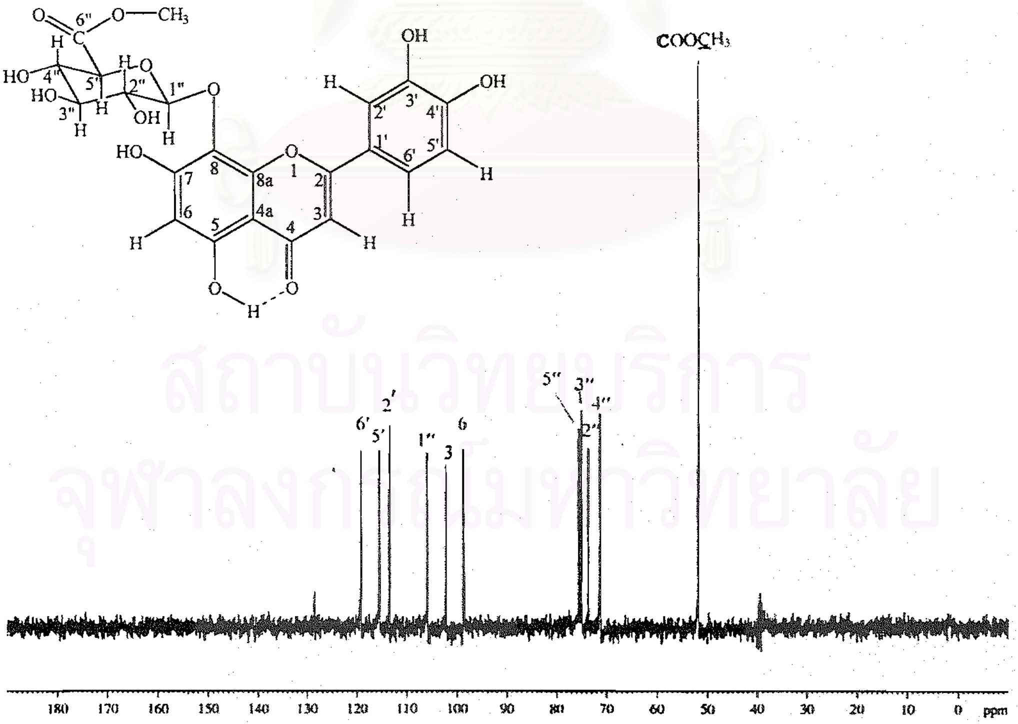


Figure 40 The 75 MHz DEPT 135 spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

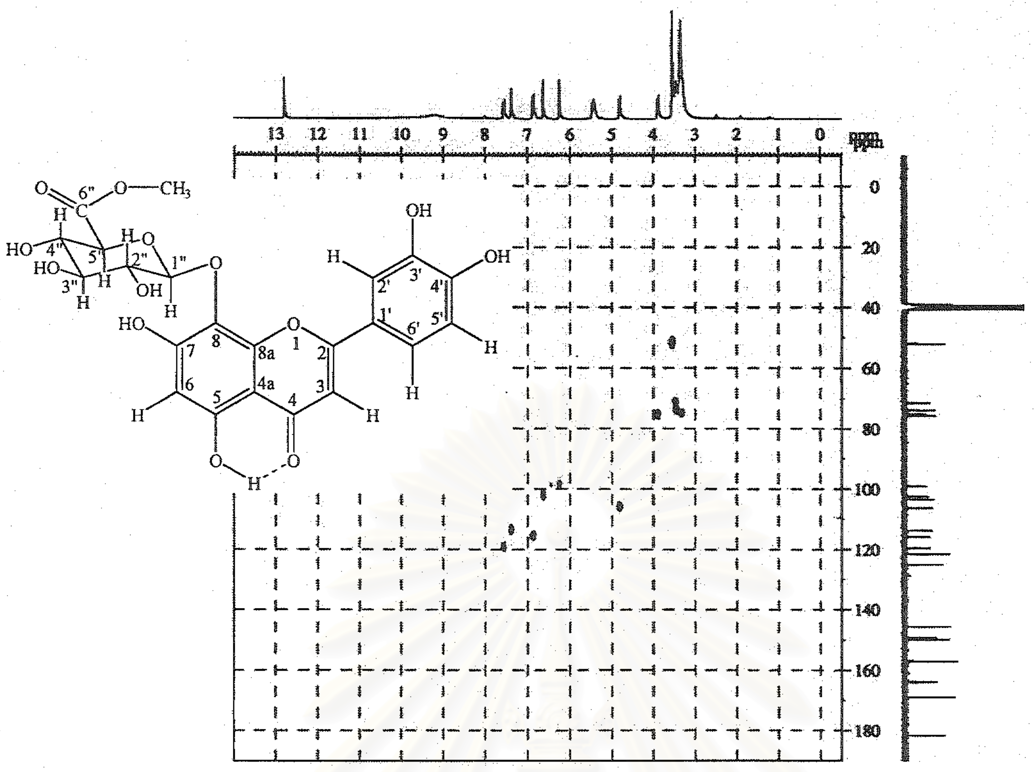


Figure 41 The 300 MHz HMQC spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

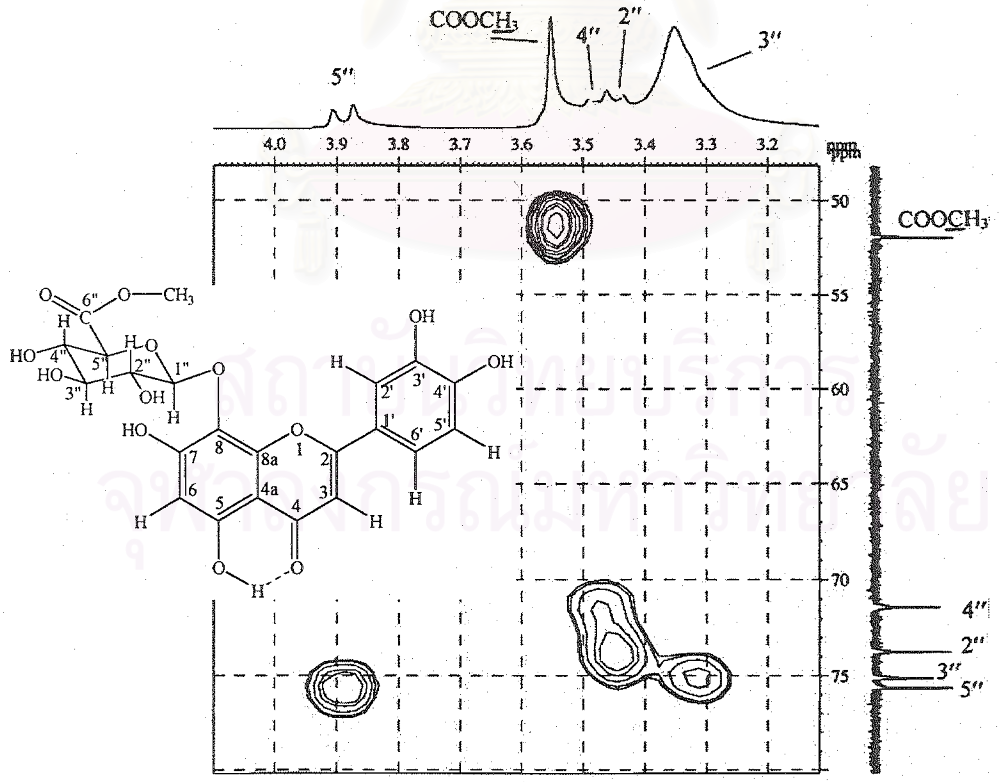


Figure 42 The 300 MHz HMQC spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2) (expanded from δ_H 3.12 - 4.10 ppm and δ_C 48.0 - 80.0 ppm)

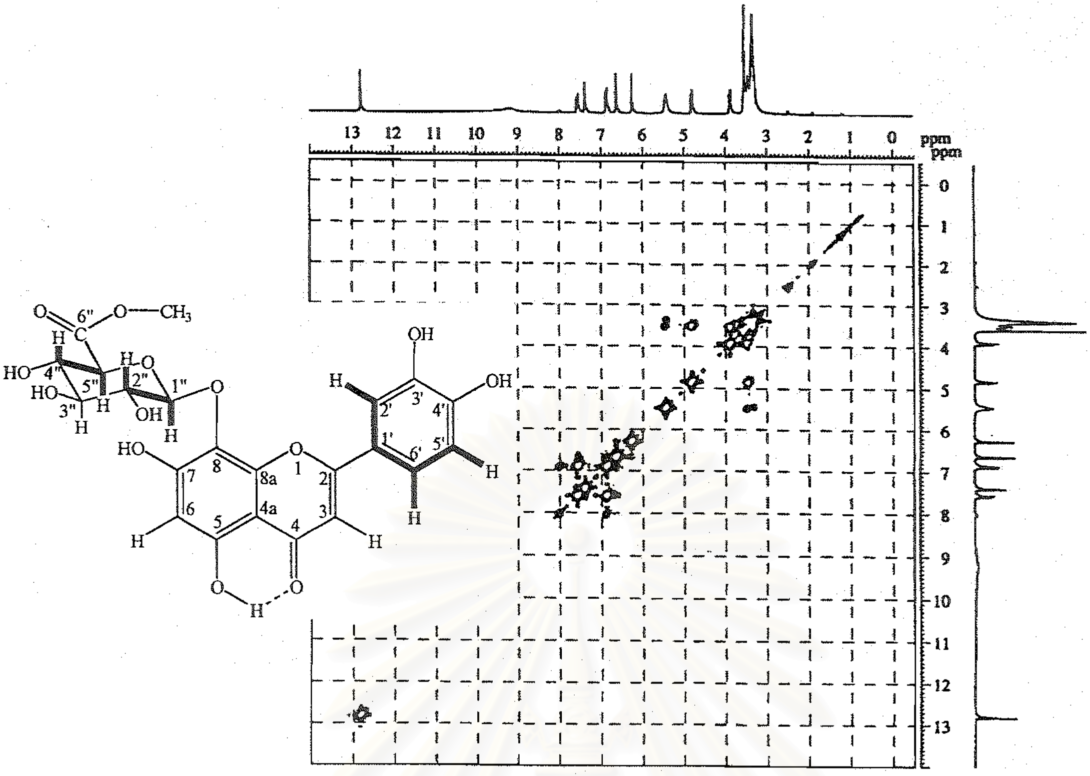


Figure 43 The 300 MHz ^{1}H-^{1}H COSY spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

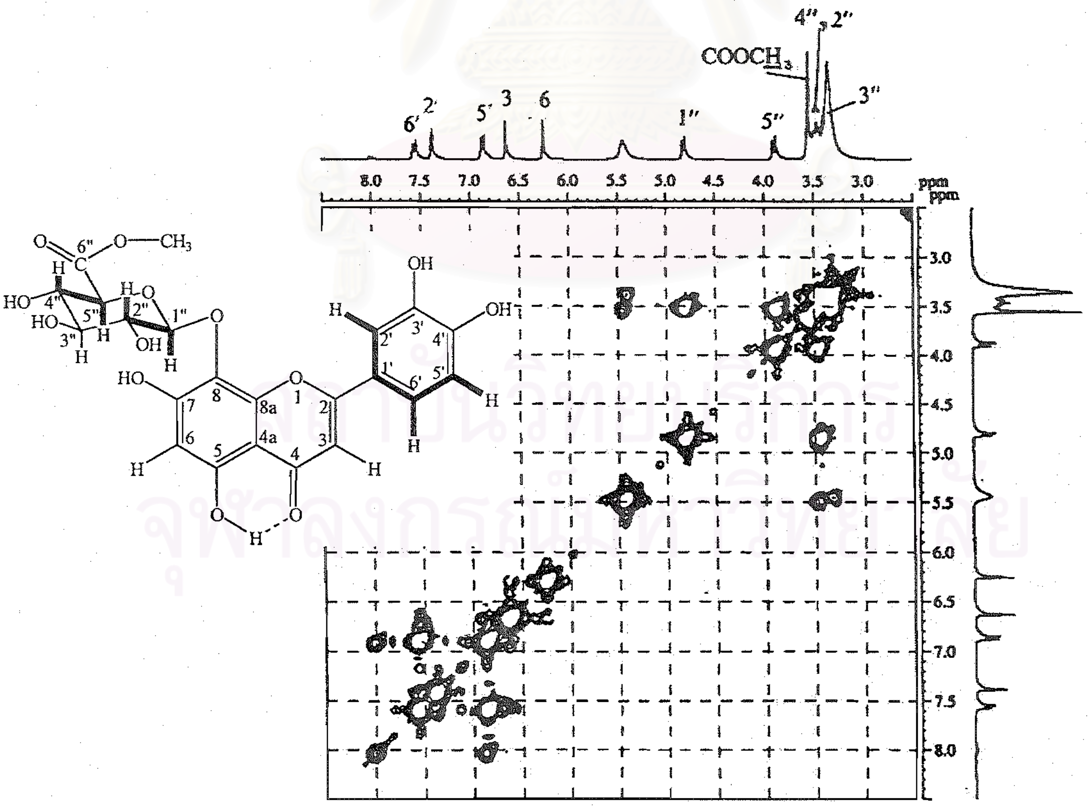


Figure 44 The 300 MHz ^{1}H-^{1}H COSY spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2) (expanded from δ_H 2.50-8.50 ppm)

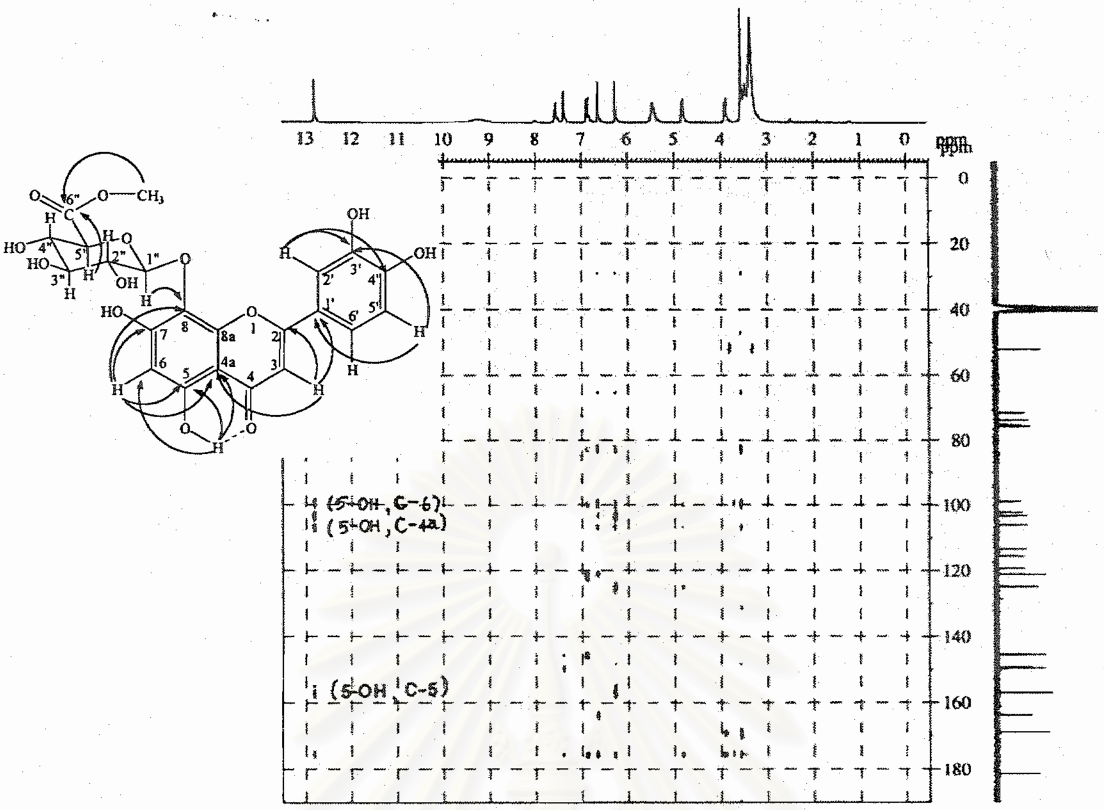


Figure 45 The HMBC spectrum ($^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

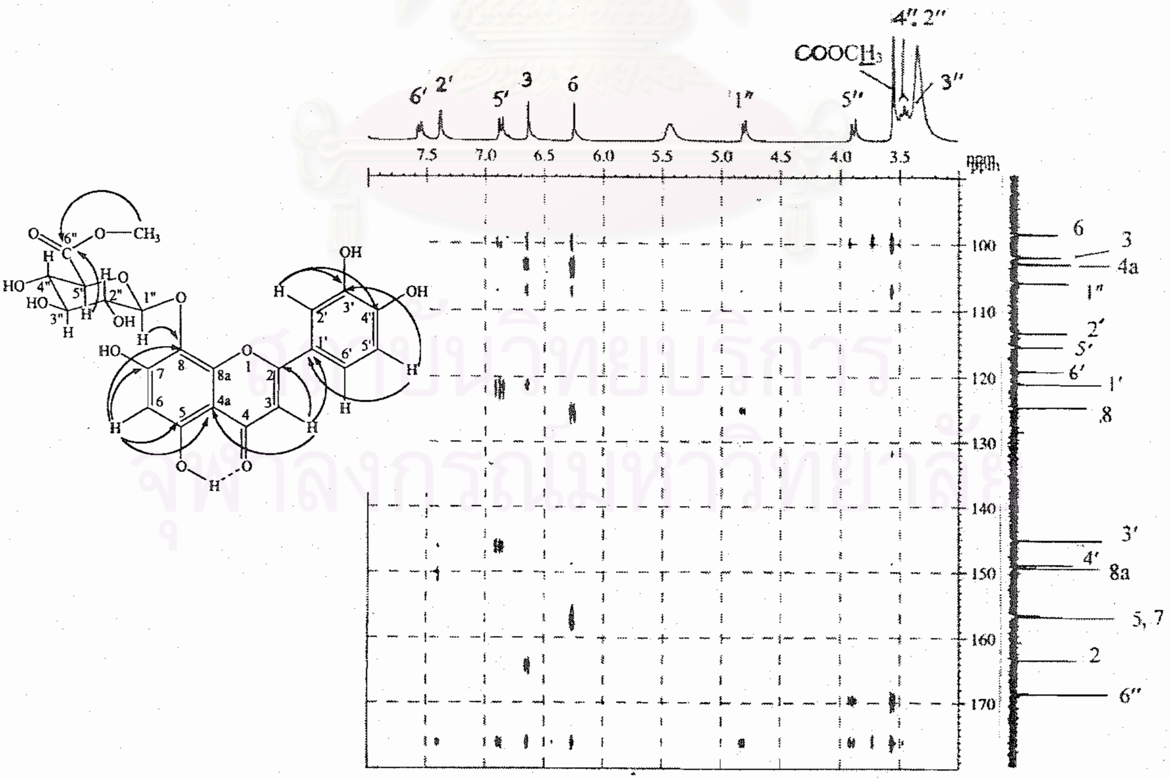


Figure 46 The 300 MHz HMBC spectrum ($^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2) (expanded from δ_H 3.00-8.00 ppm and δ_C 90.0-180.0 ppm)

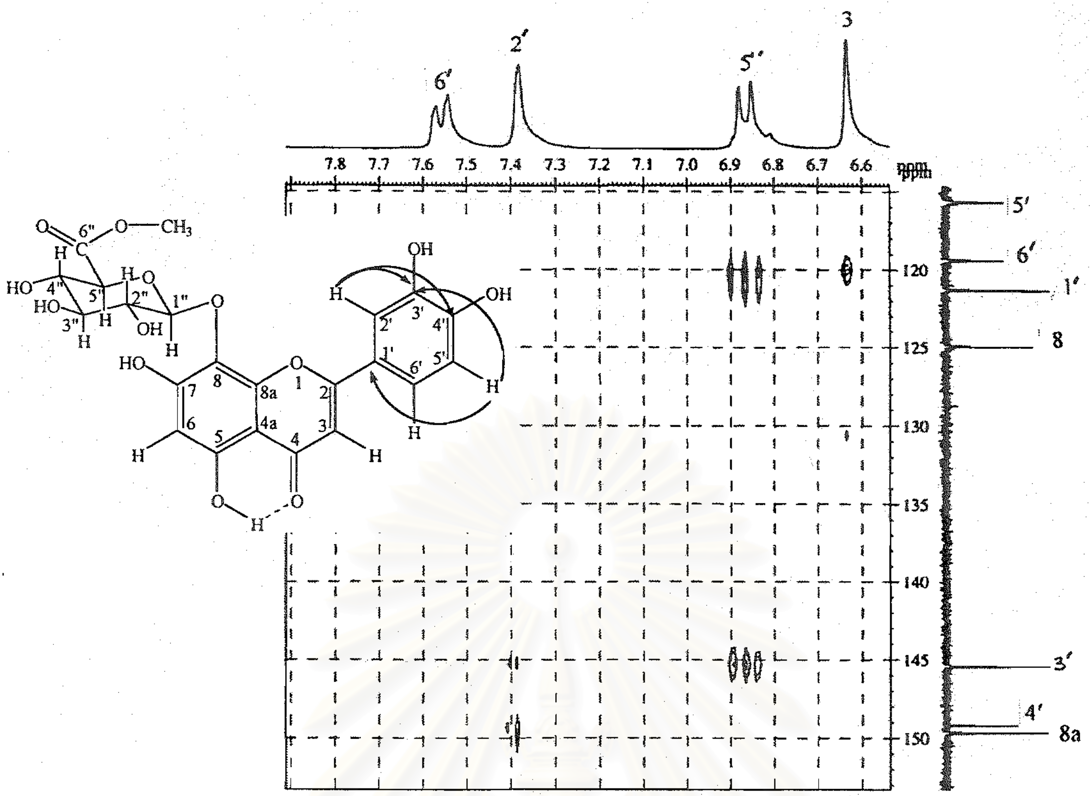


Figure 47 The 300 MHz HMBC spectrum ($^nJ_{HC}$ = 8 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2) (expanded from δ_H 6.54-7.91 ppm and δ_C 110.0-153.0 ppm)

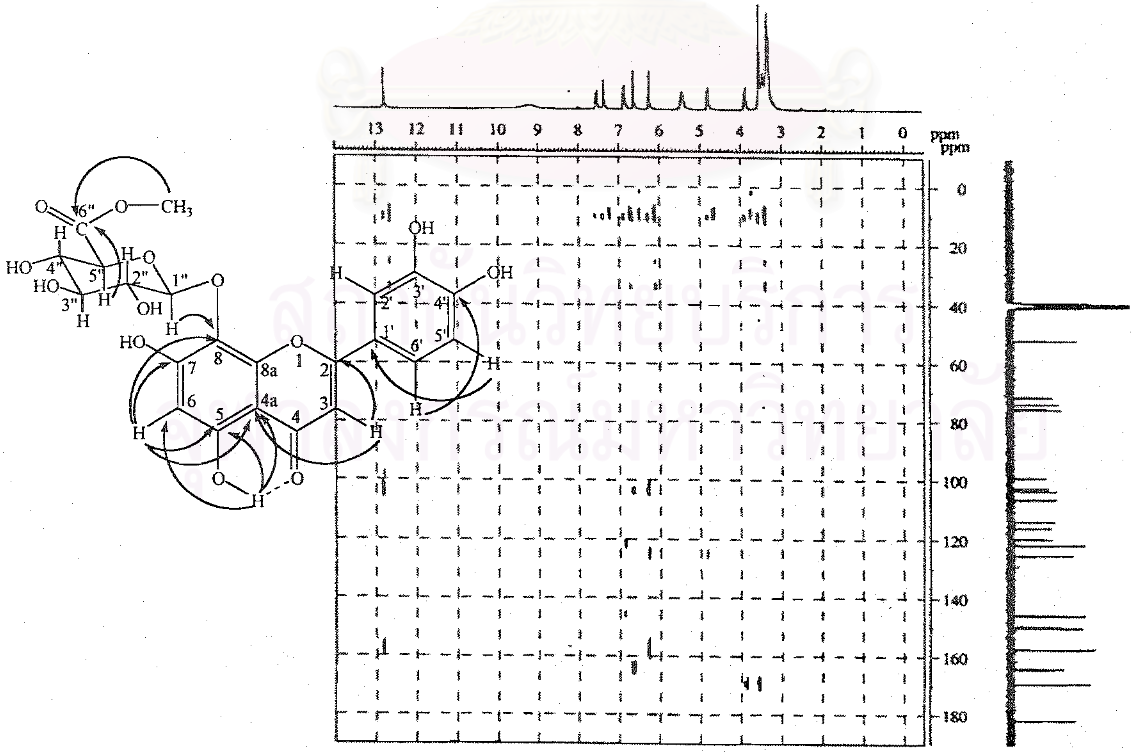


Figure 48 The 300 MHz HMBC spectrum ($^nJ_{HC}$ = 4 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside methyl ester (PL-2)

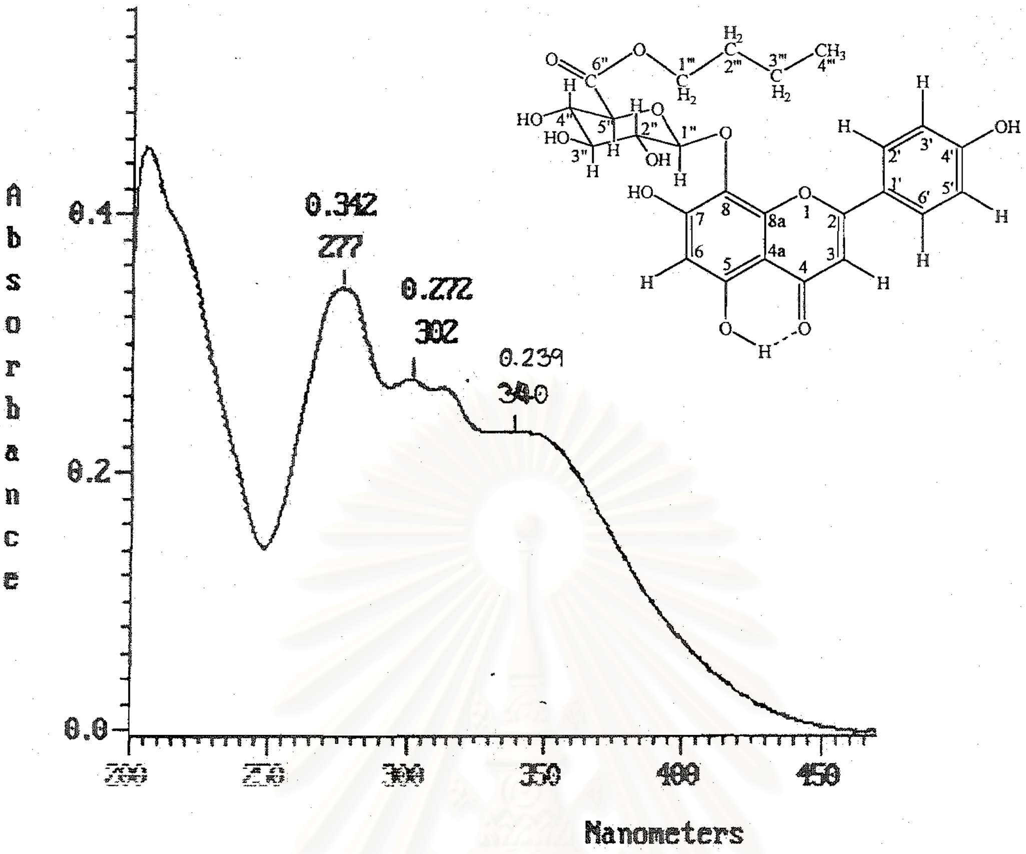


Figure 49 The UV spectrum (in MeOH) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

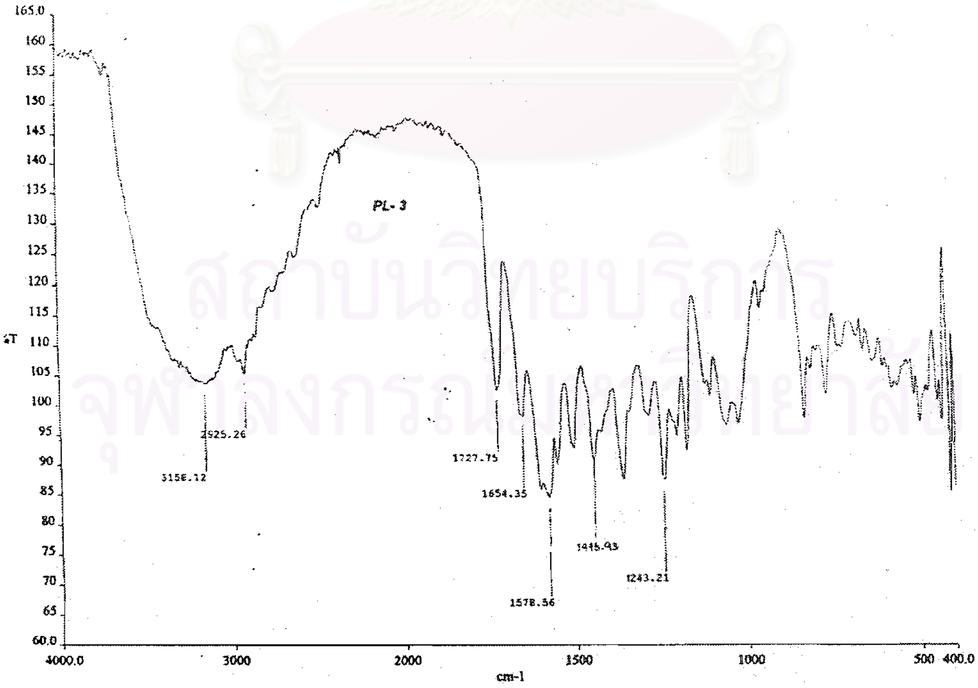


Figure 50 The IR spectrum (in KBr disc) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

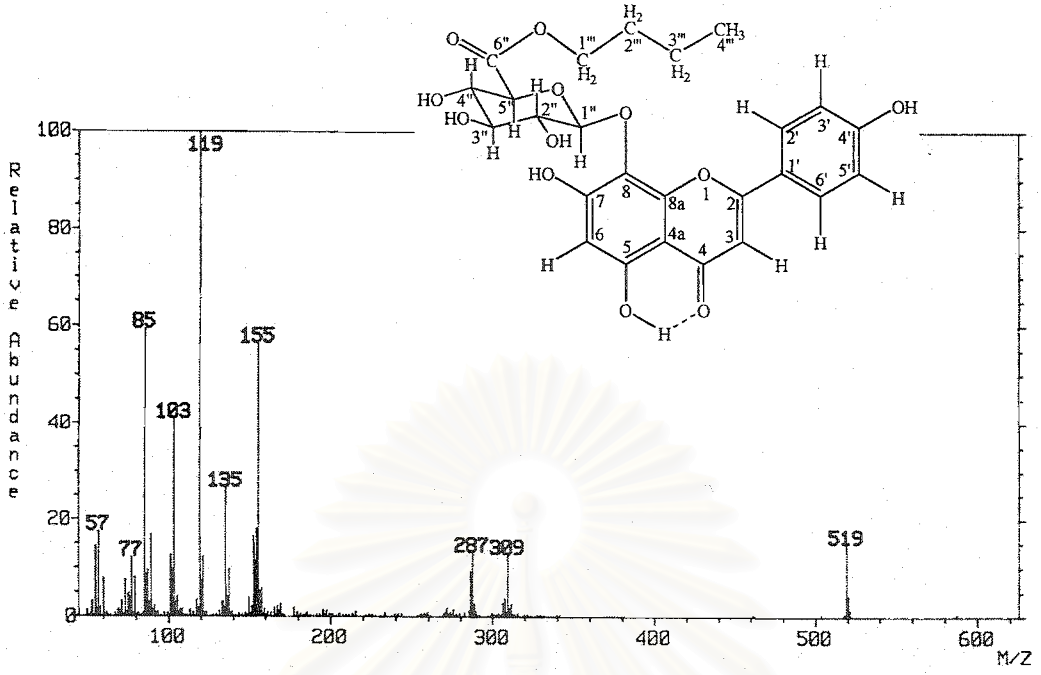


Figure 51 The FABMS spectrum of isoscutellarein8-O-β-D-glucuronopyranoside butyl ester (PL-3)

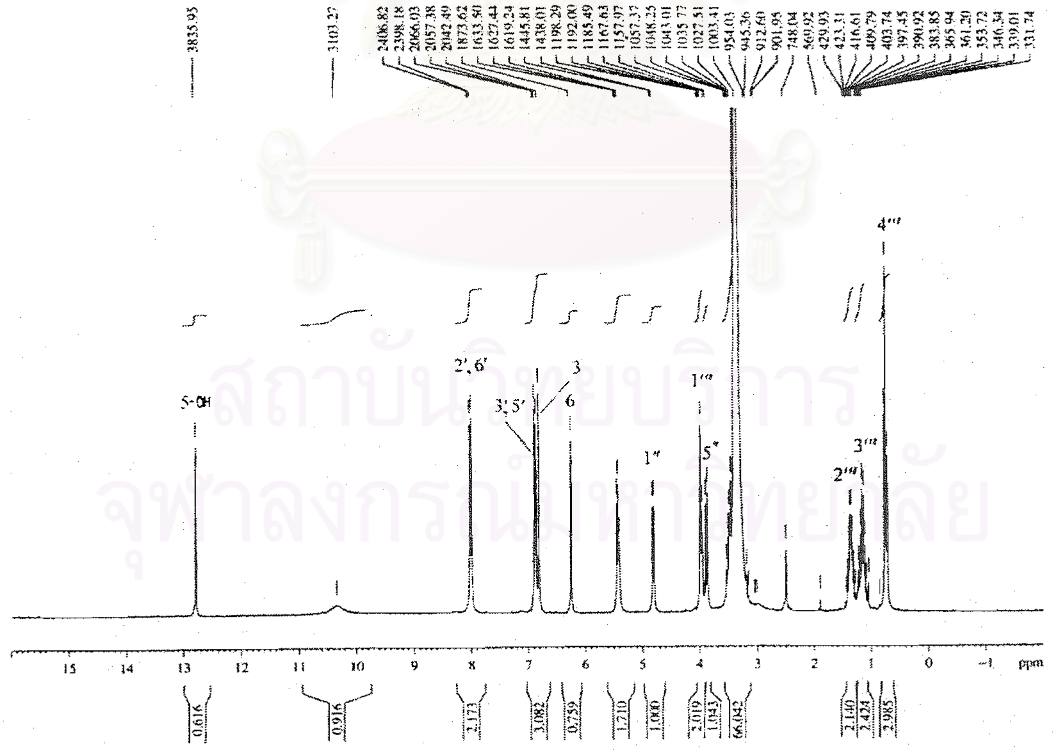


Figure 52 The 300 MHz ^{1}H NMR spectrum (in DMSO-d_6) of isoscutellarein 8-O-β-D-glucuronopyranoside butyl ester (PL-3)

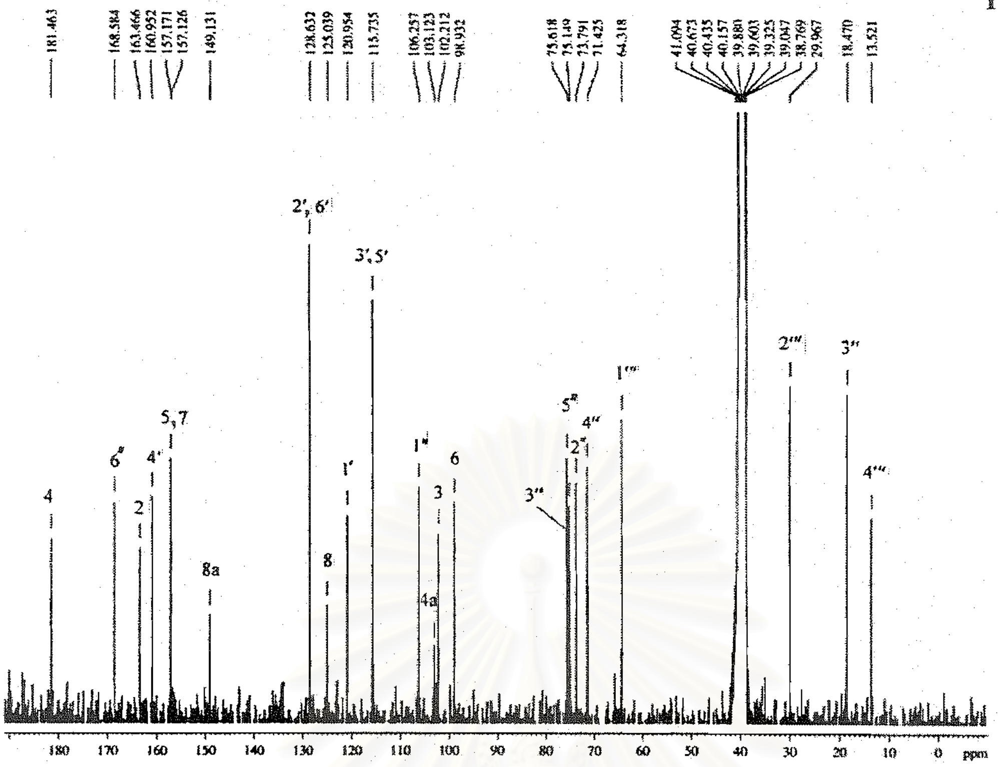
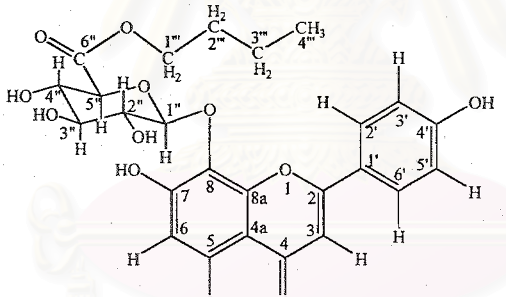


Figure 53 The 75 MHz ^{13}C NMR spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)



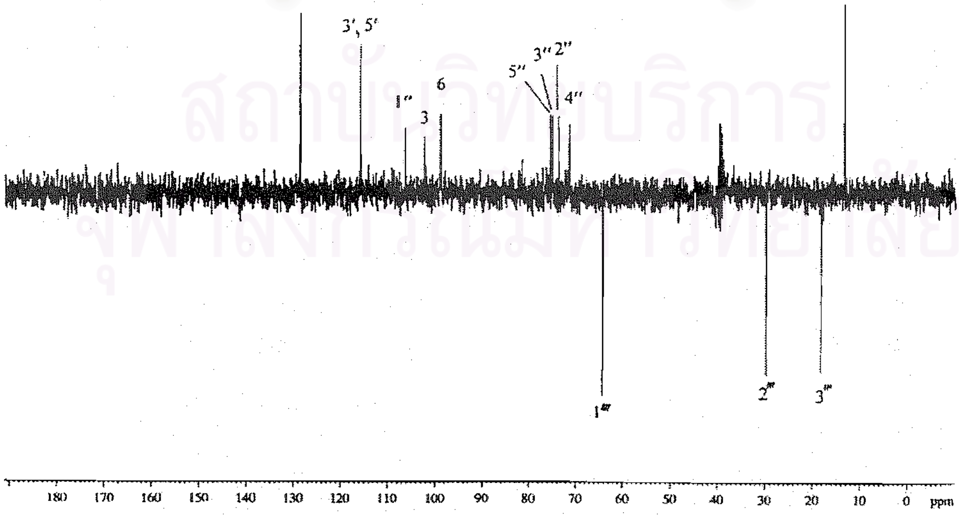


Figure 54 The 75 MHz DEPT 135 spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

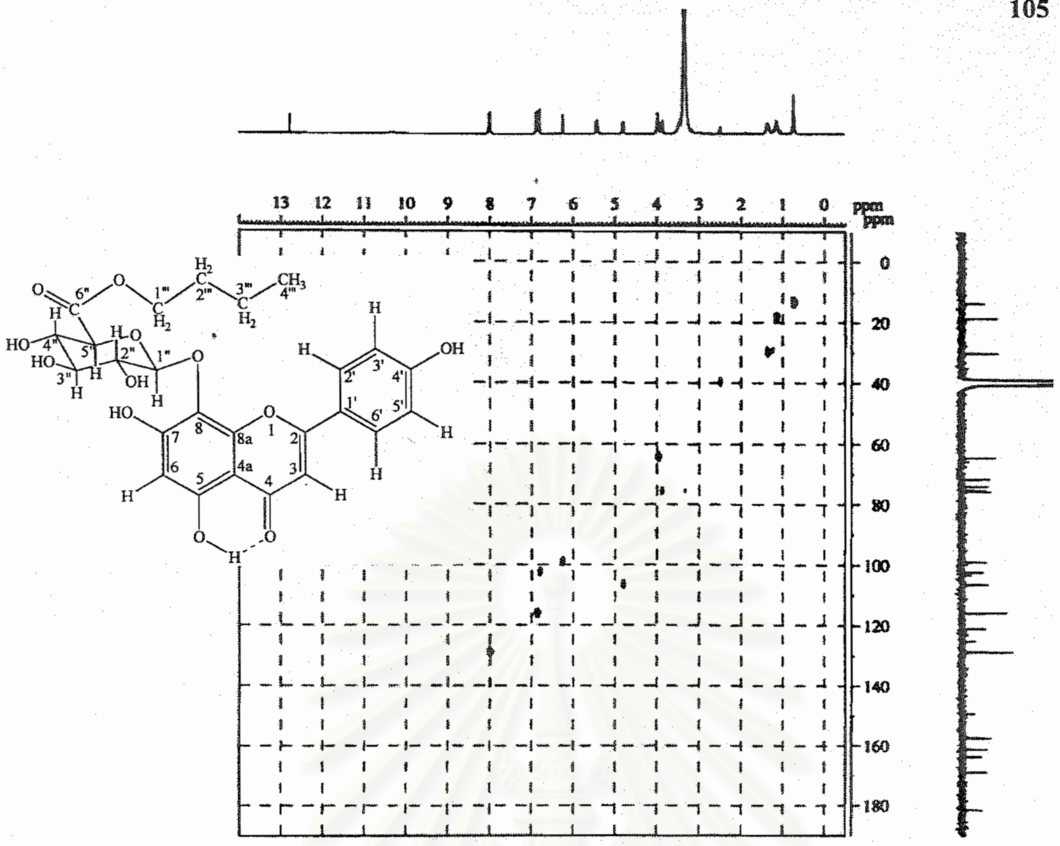


Figure 55 The 300 MHz HMQC spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

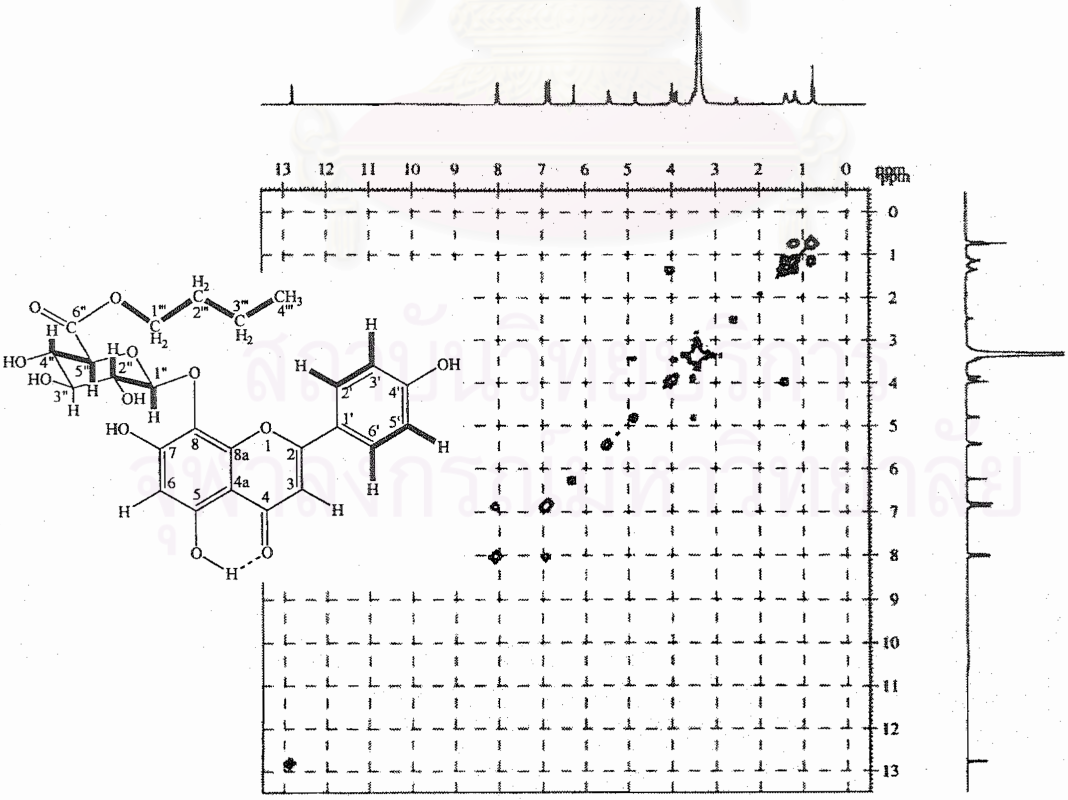


Figure 56 The 300 MHz ^{1}H-^{1}H COSY spectrum (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

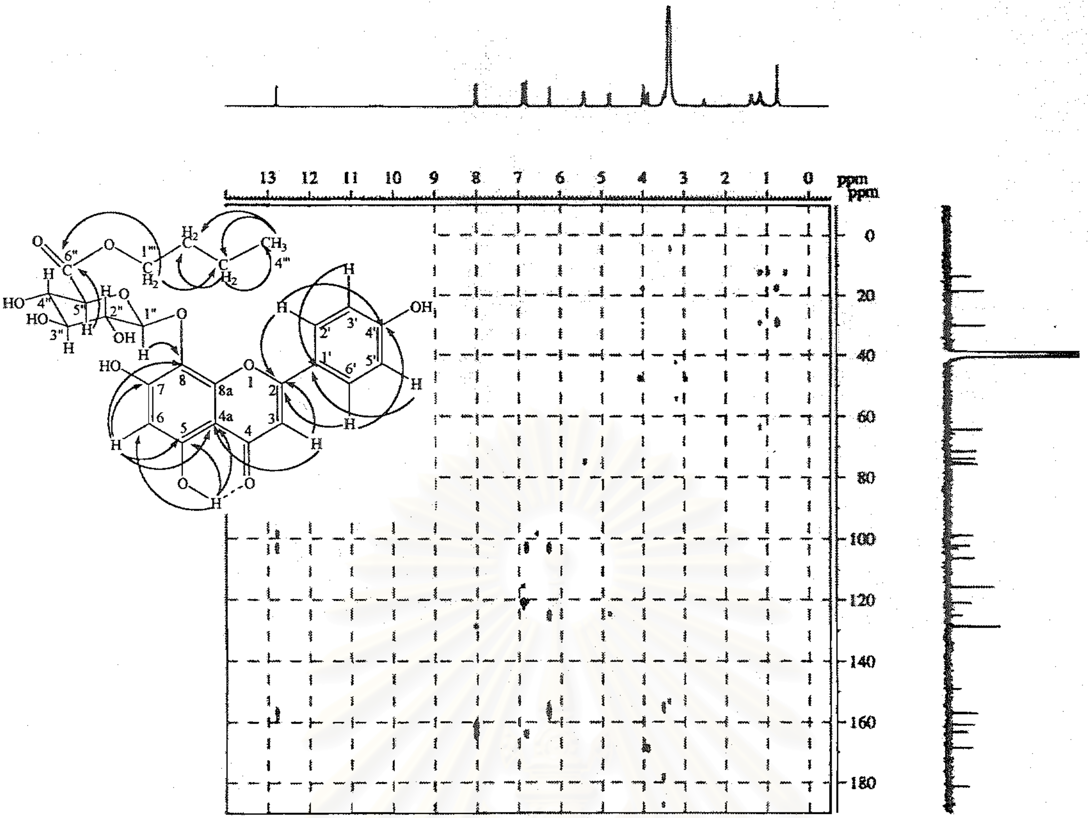


Figure 57 The HMBC spectrum (${}^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

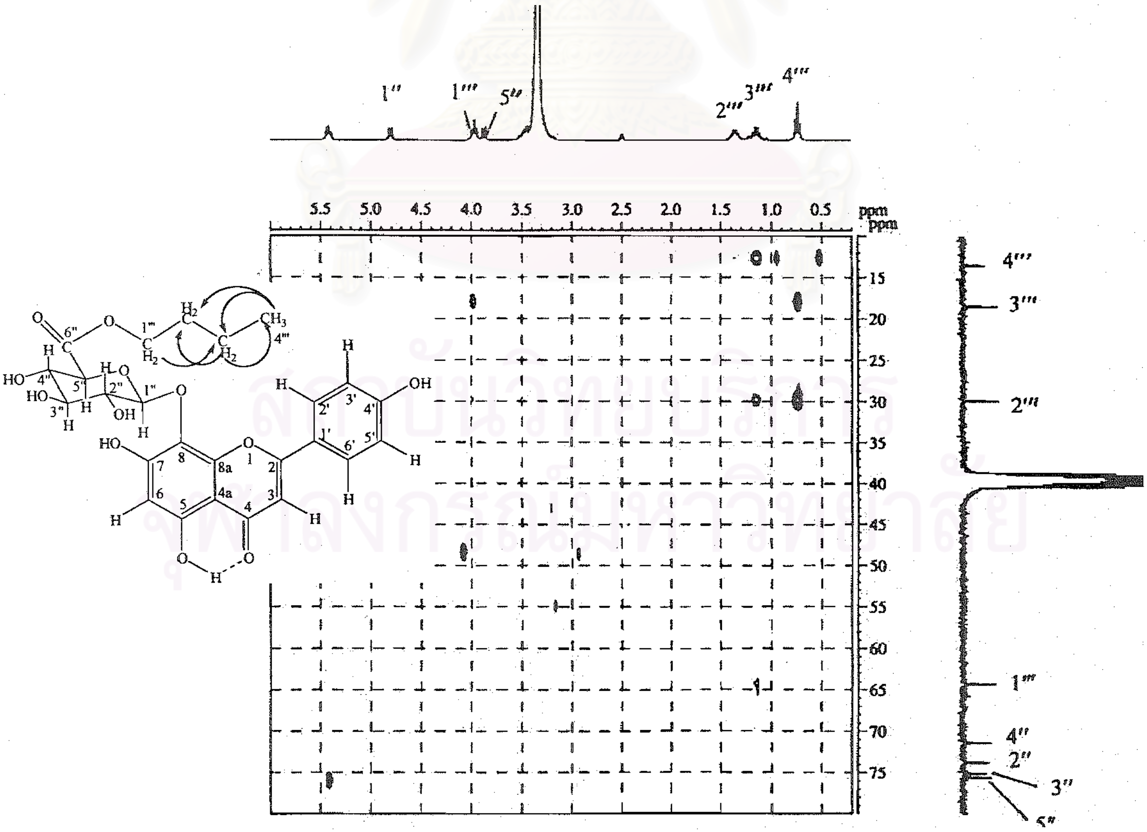


Figure 58 The 300 MHz HMBC spectrum (${}^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3) (expanded from δ_H 0.20-6.00 ppm and δ_C 10.0-80.0 ppm)

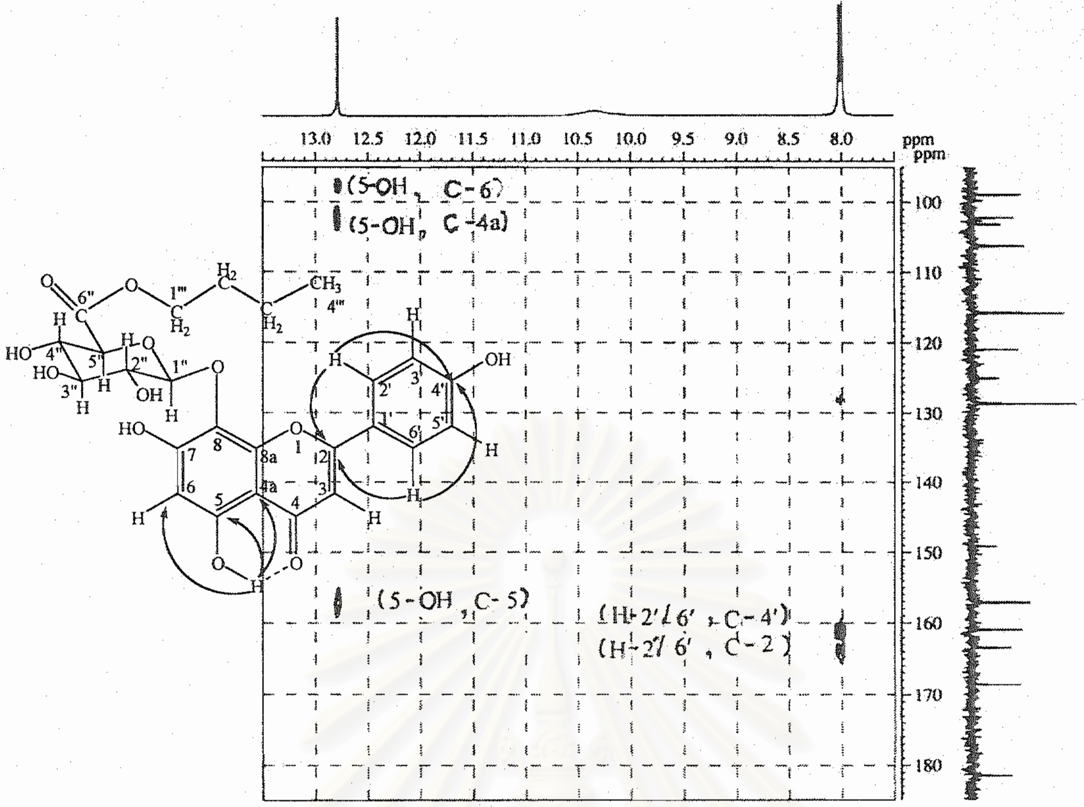


Figure 59 The 300 MHz HMBC spectrum ($^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3) (expanded from δ_H 7.50-13.50 ppm and δ_C 95.0-185.0 ppm)

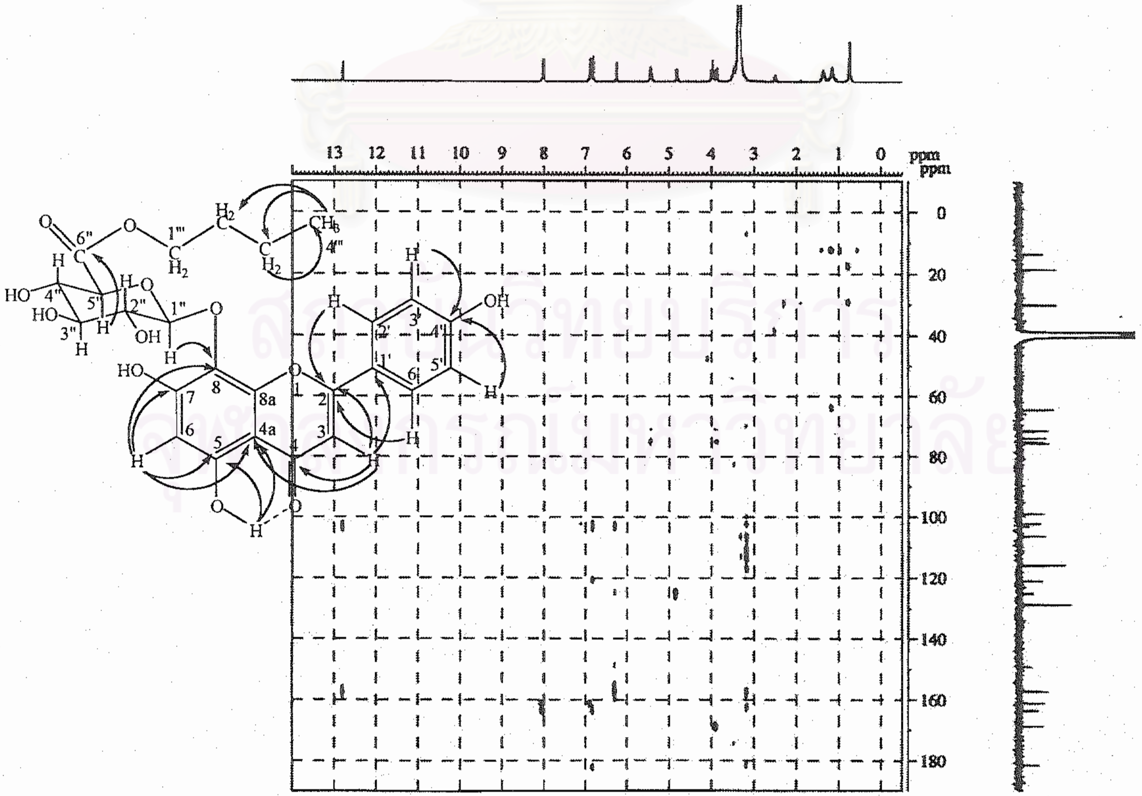


Figure 60 The 300 MHz HMBC spectrum ($^{n}J_{HC}$ = 4 Hz) (in DMSO-d_6) of isoscutellarein 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-3)

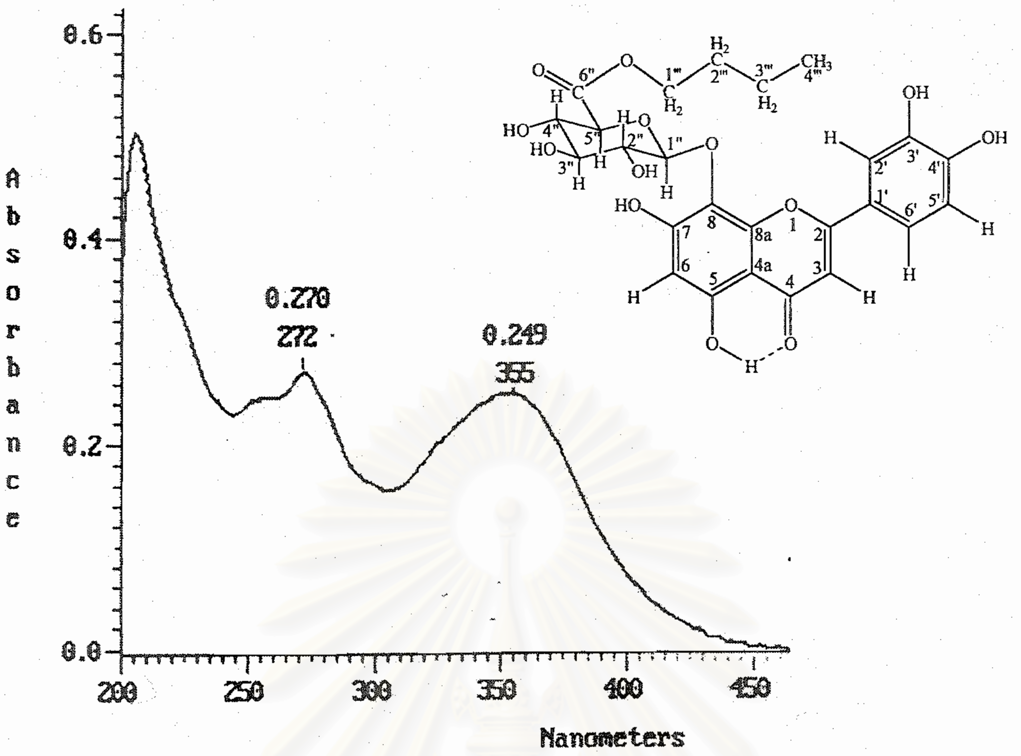


Figure 61 The UV spectrum (in MeOH) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

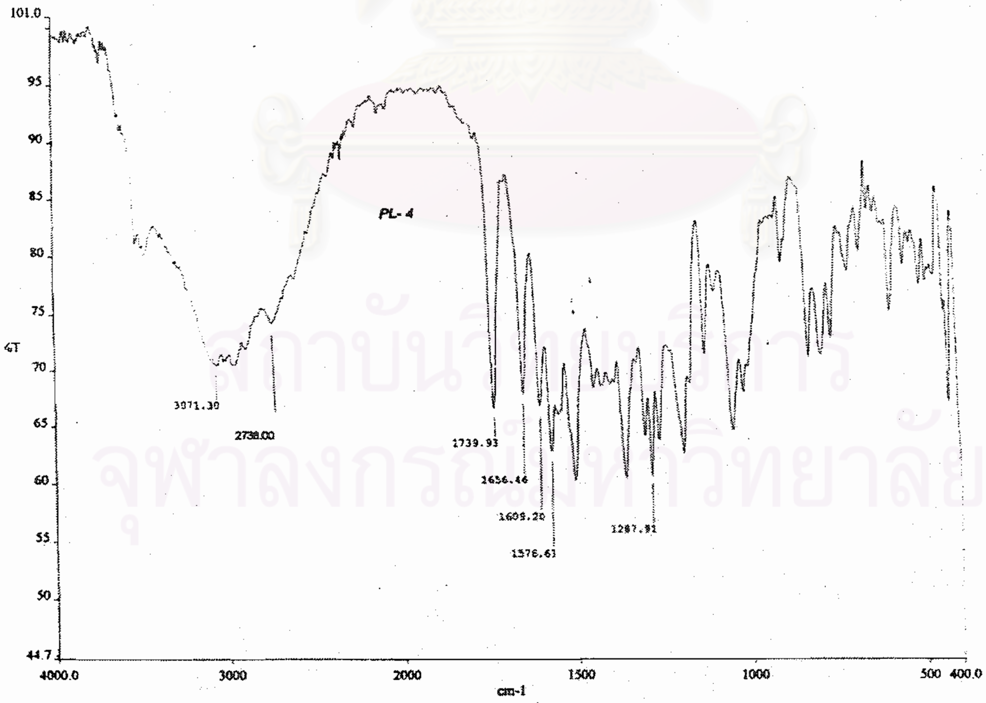


Figure 62 The IR spectrum (in KBr disc) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

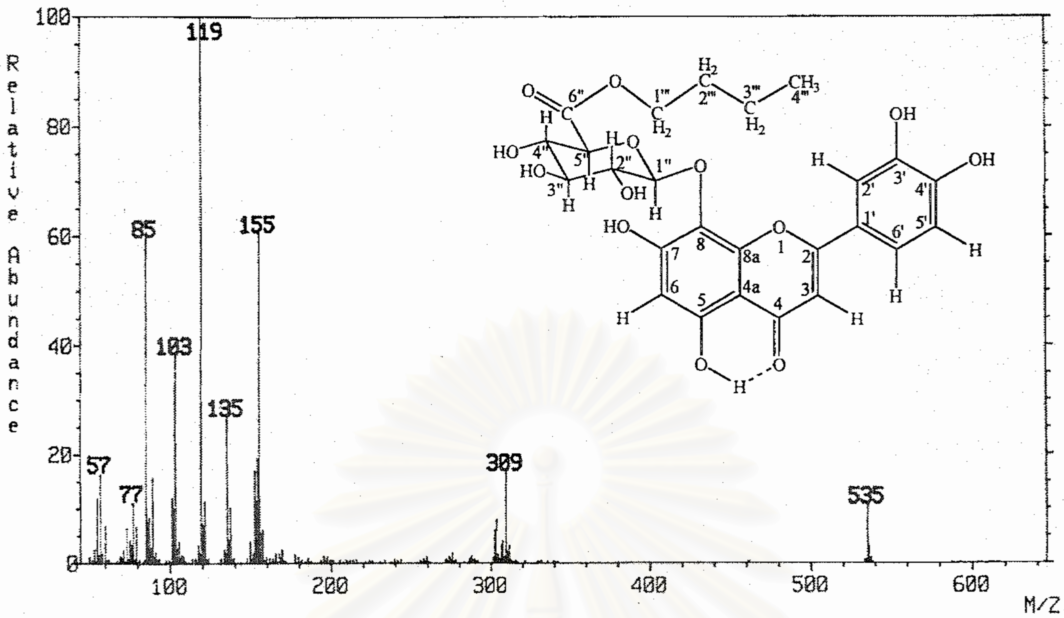


Figure 63 The FABMS spectrum of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

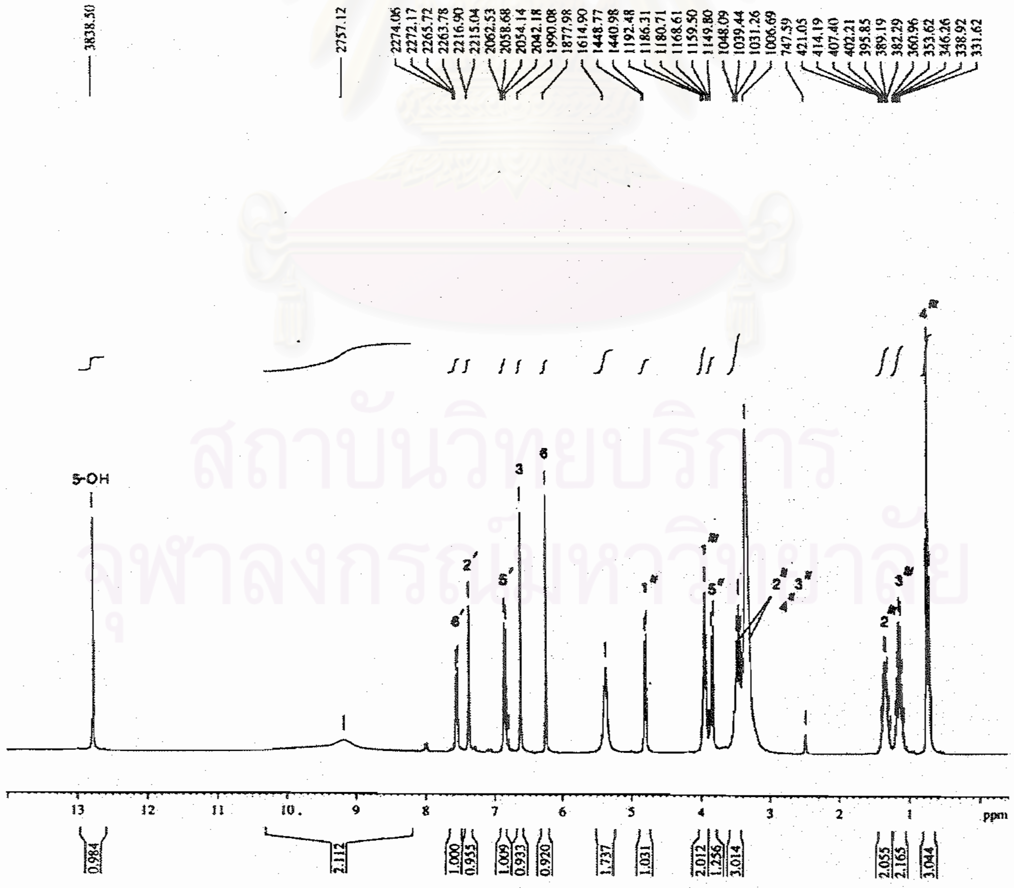


Figure 64 The 300 MHz ^{1}H NMR spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

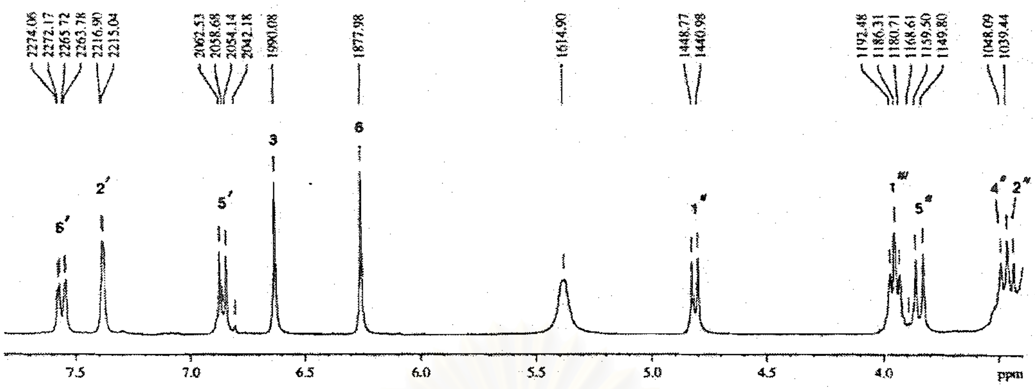


Figure 65 The 300 MHz ^{1}H NMR spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4) (expanded from δ_H 3.40-7.80 ppm

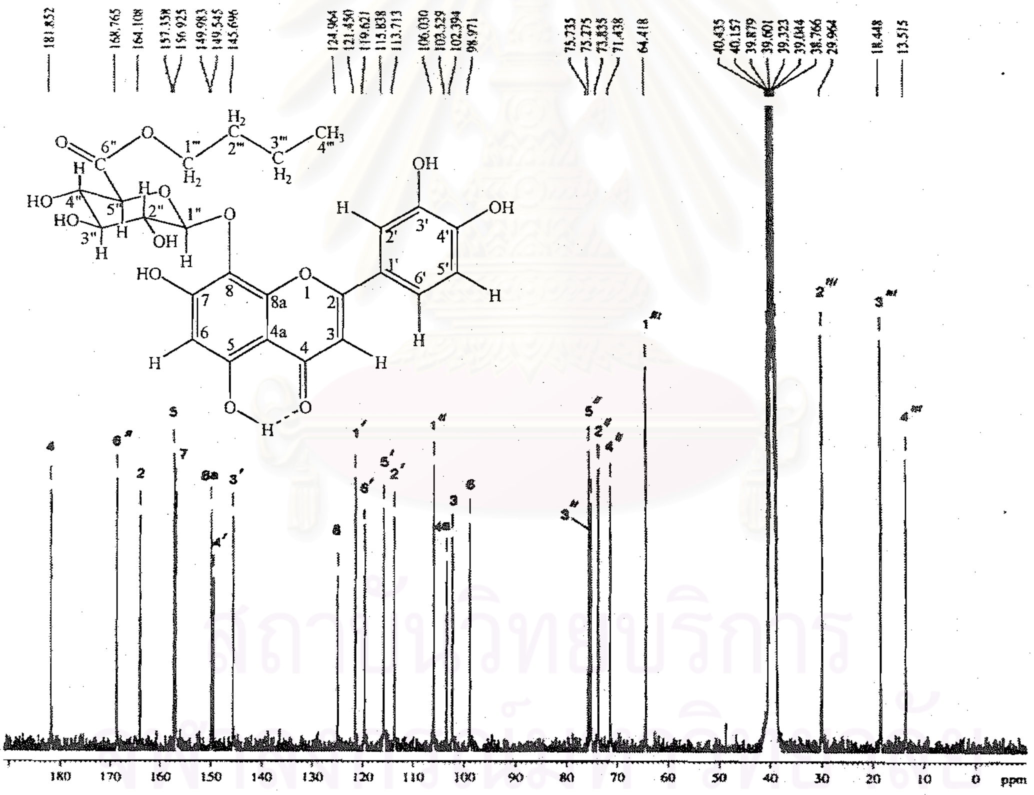


Figure 66 The 75 MHz ^{13}C NMR spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

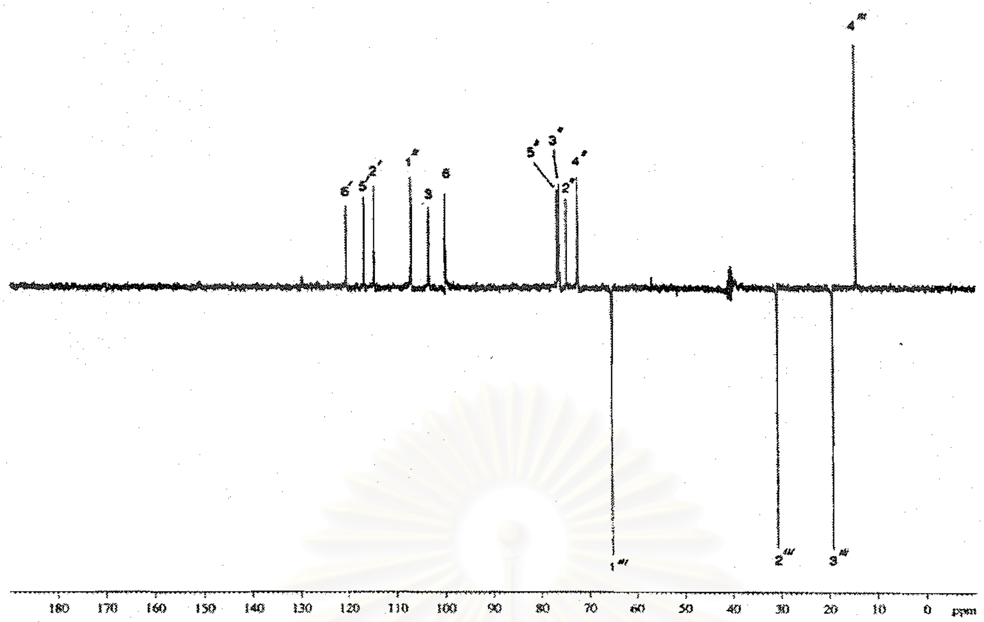


Figure 67 The 75 MHz DEPT 135 spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

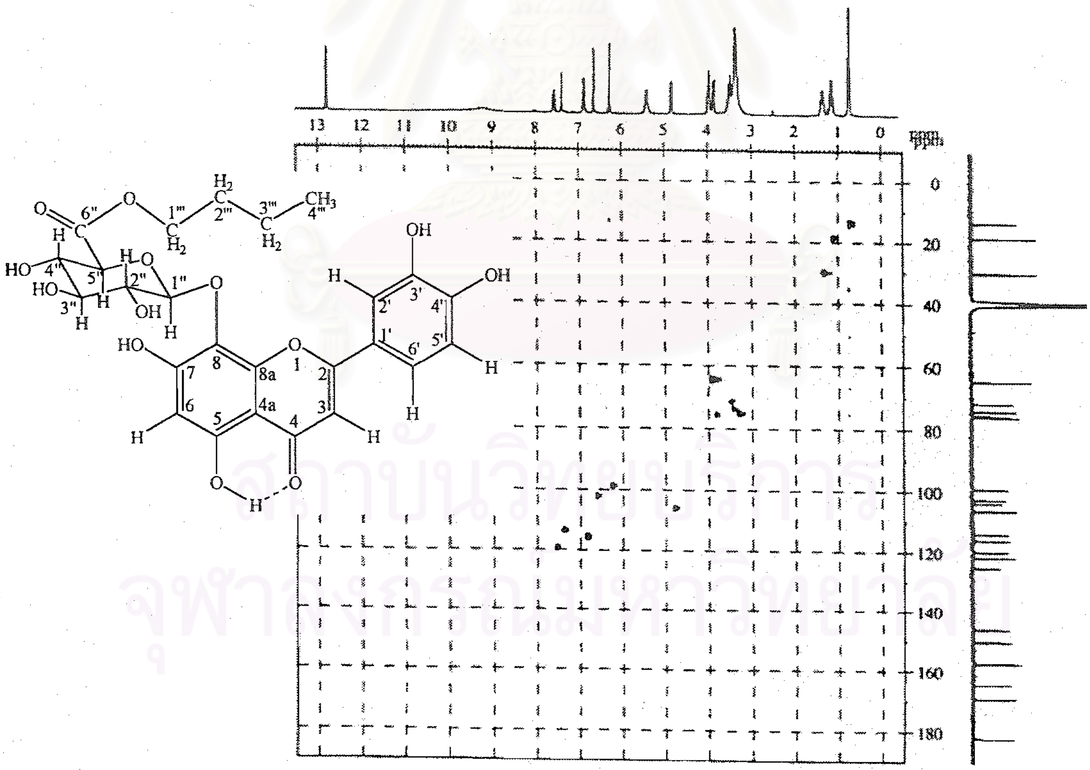


Figure 68 The 300 MHz HMQC spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

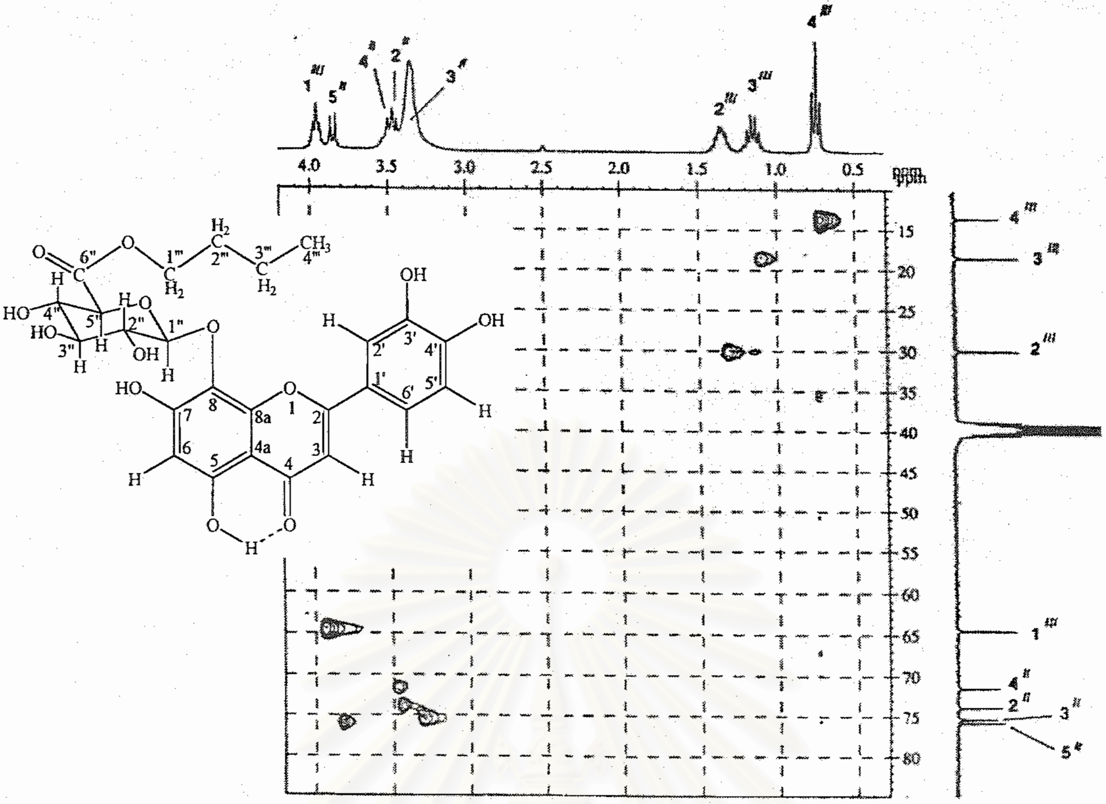


Figure 69 The 300 MHz HMQC spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4) (expanded from δ_H 0.30 - 4.20 ppm and δ_C 10.0 - 85.0 ppm)

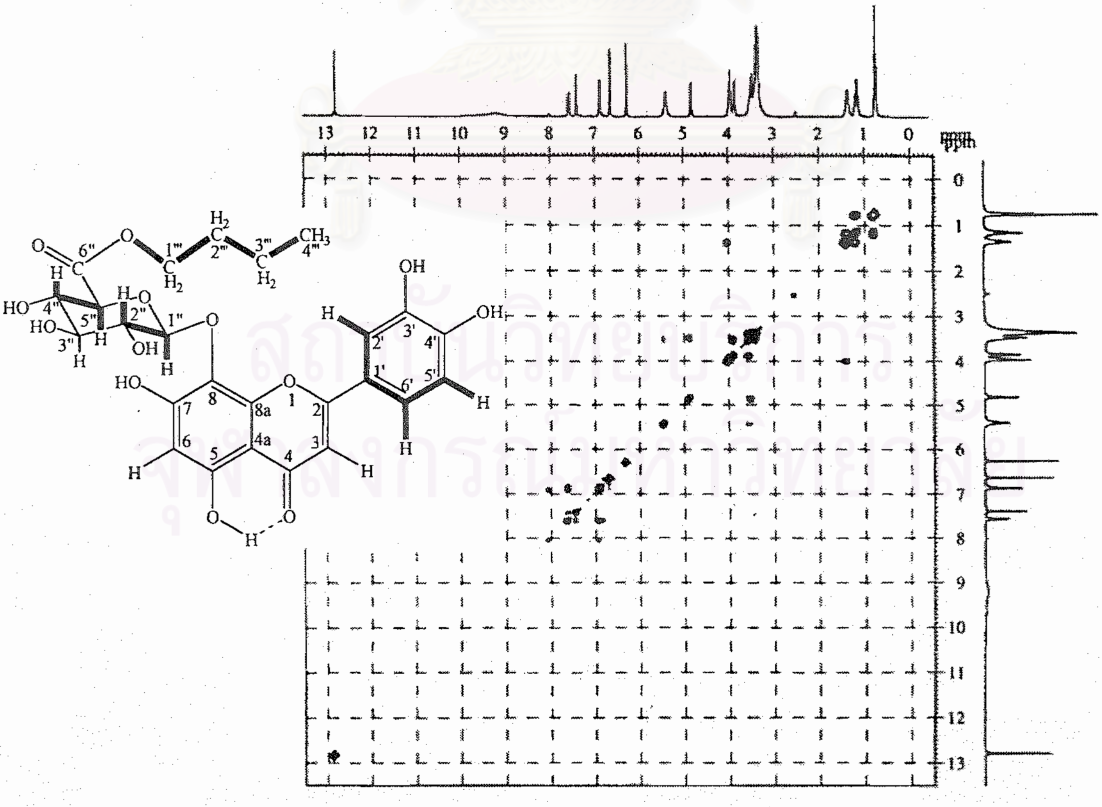


Figure 70 The 300 MHz ^{1}H-^{1}H COSY spectrum (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

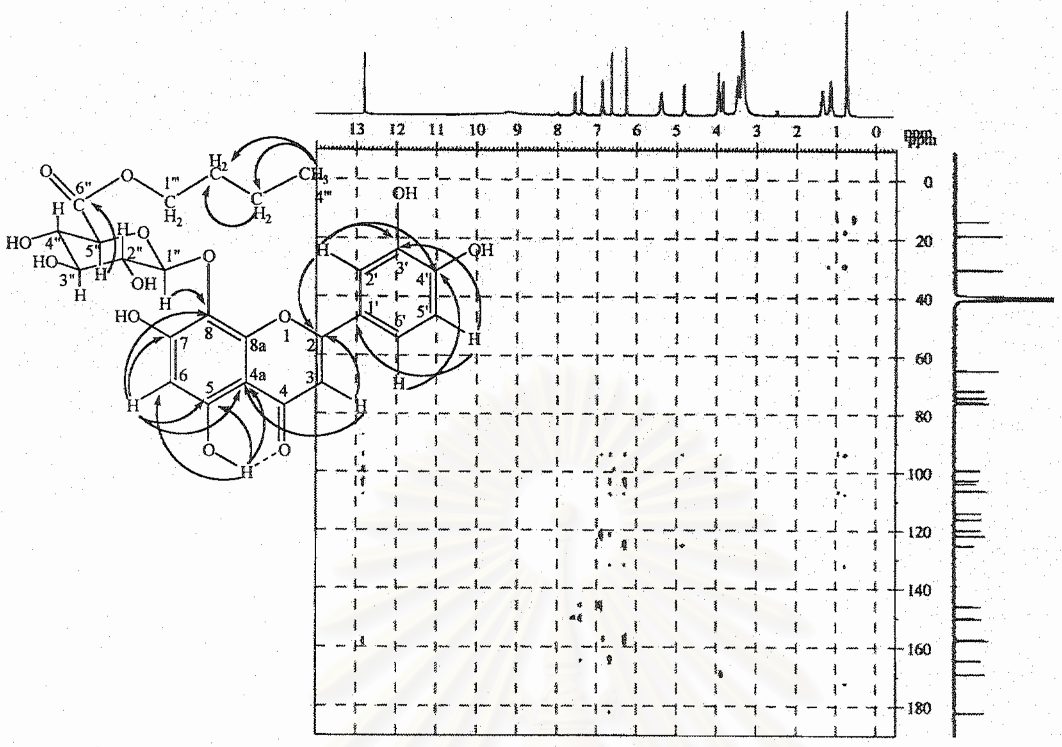


Figure 71 The 300 MHz HMBC spectrum (J_{HC} = 8 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

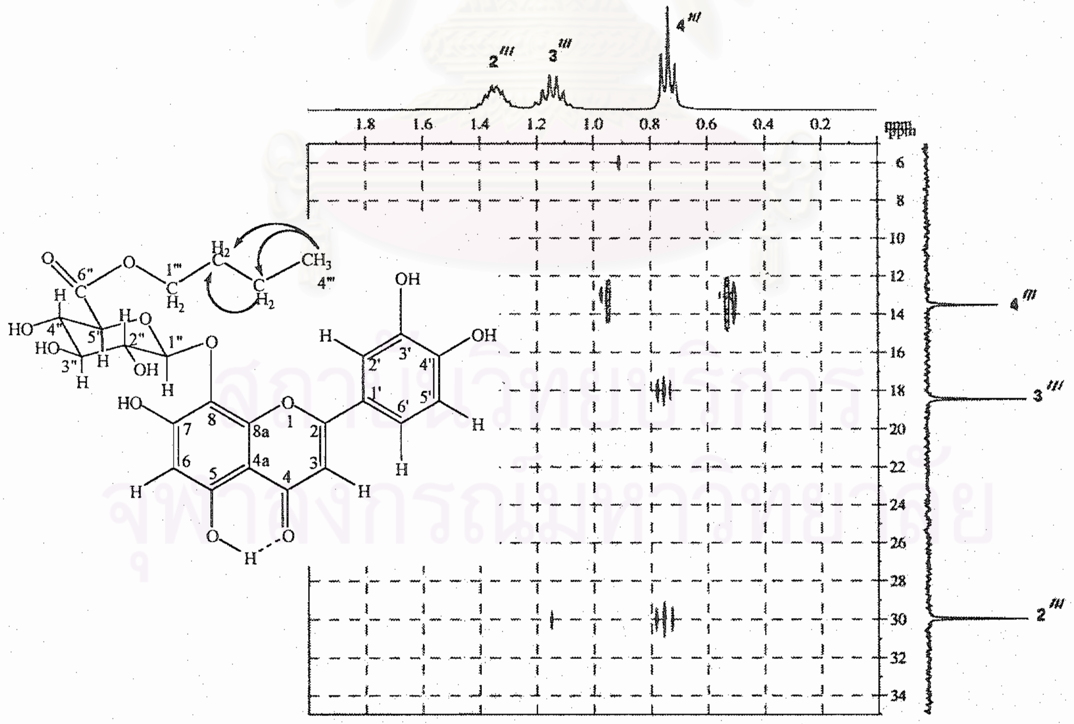


Figure 72 The HMBC spectrum ($^nJ_{HC}$= 8 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4) (expanded from δ_H 0.00 – 2.00 ppm and δ_C 5.0-35.0 ppm)

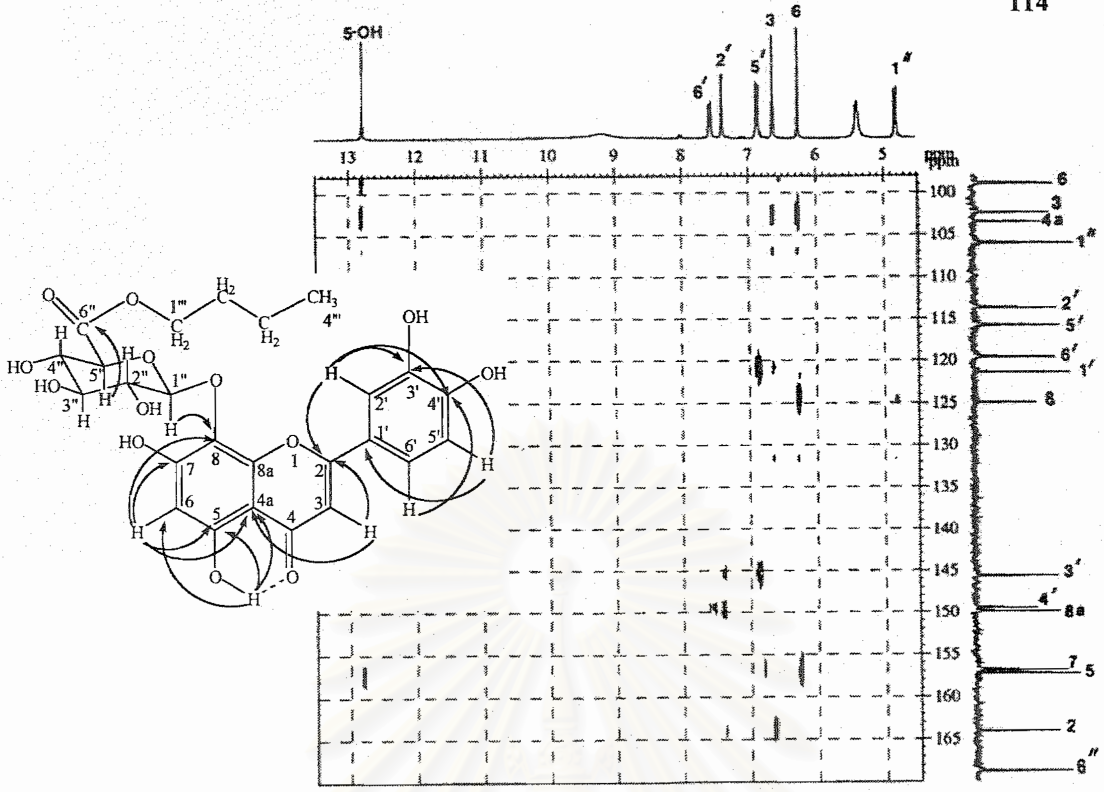


Figure 73 The 300 MHz HMBC spectrum ($^{n}J_{HC}$ = 8 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4) (expanded from δ_H 4.50-13.50 ppm and δ_C 98.0-170.0 ppm)

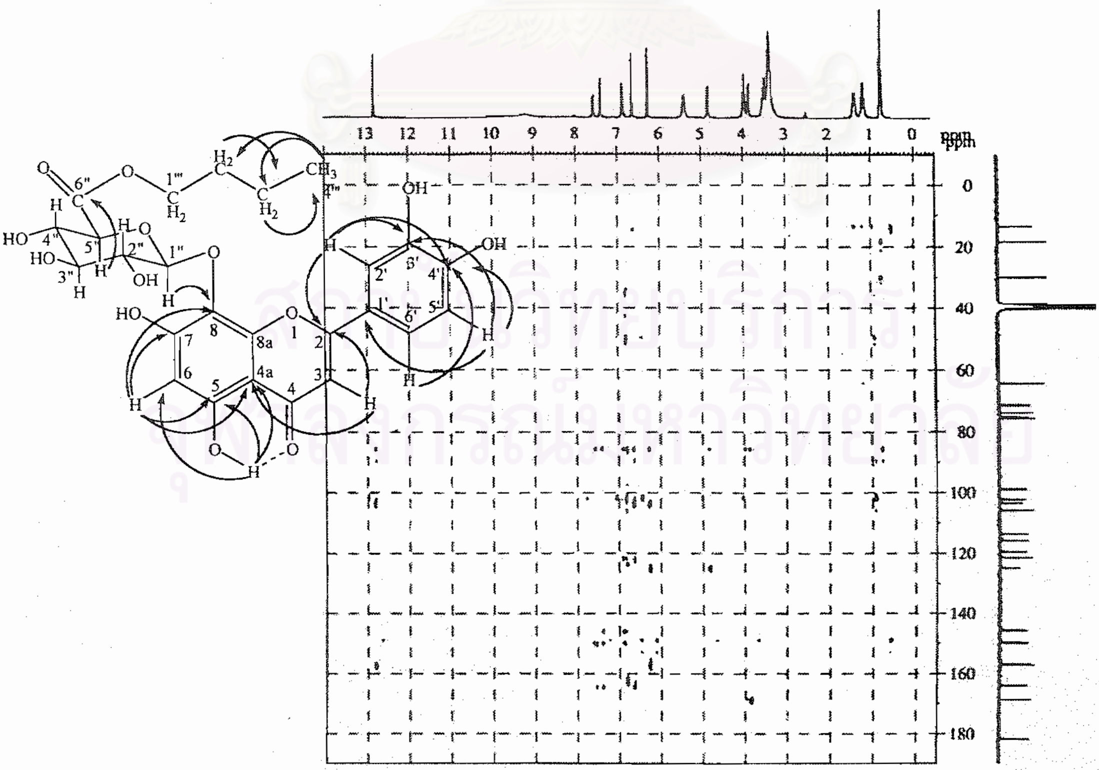


Figure 74 The 300 MHz HMBC spectrum ($^{n}J_{HC}$ = 4 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4)

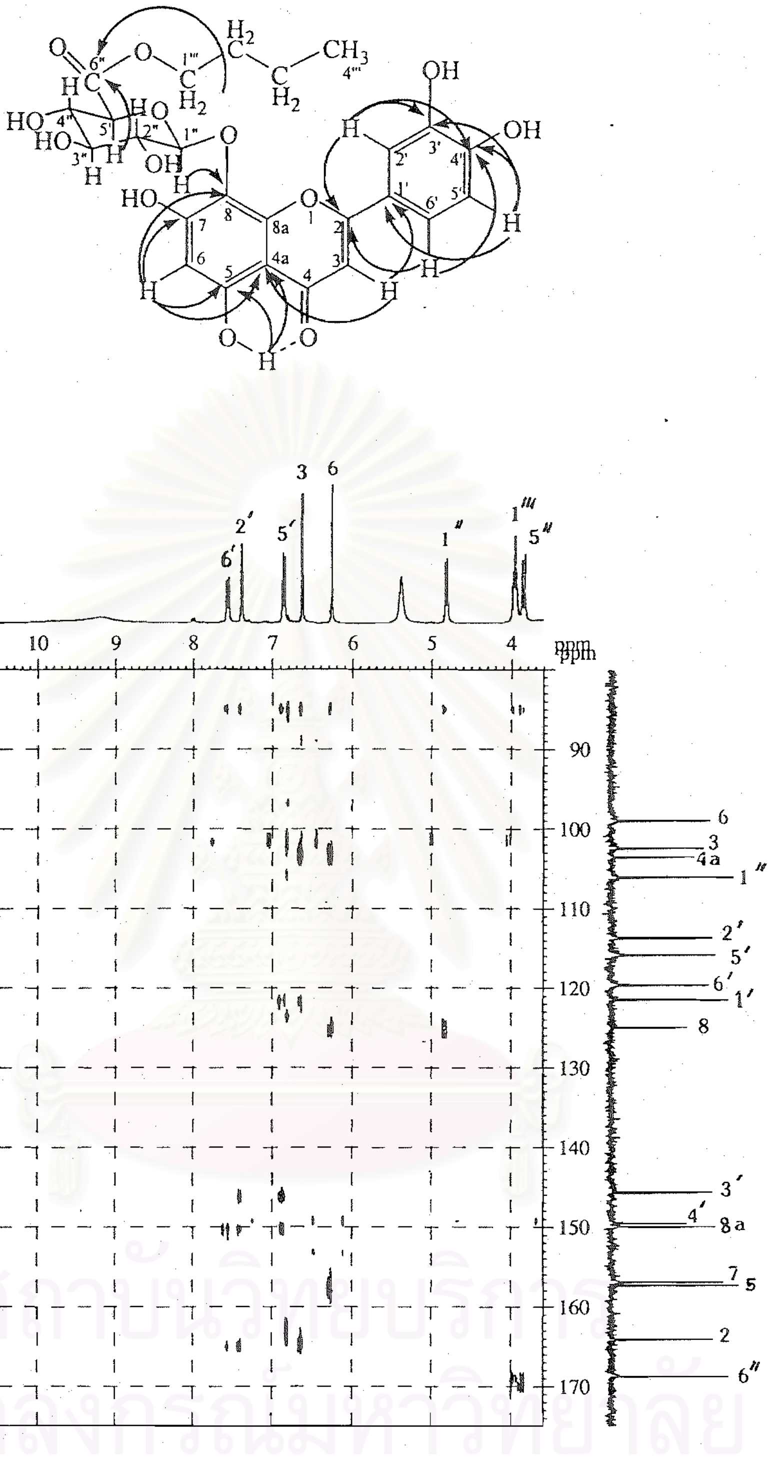


Figure 75 The 300 MHz HMBC spectrum ($^{n}J_{HC}$ = 4 Hz) (in DMSO-d_6) of hypolaetin 8-*O*-*β*-D-glucuronopyranoside butyl ester (PL-4) (expanded from δ_H 3.60 – 13.20 ppm and δ_C 80.0 – 175.0 ppm)

VITA

Miss Bongkot Phanburananont was born on April 7, 1973 in Phetchaburi, Thailand. She received her Bachelor Degree of Science in Pharmacy in 1996 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Since graduation, she has been working as a pharmacist in the Narcotic Division, Medical Sciences Department, Thailand.