สารยับยั้งไซโคลออกซิเจนเนส-2 จากแก่นจันทน์แดง

นางสาว กนกภรณ์ สวัสดี

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INHIBITORS OF CYCLOOXYGENASE-2 FROM *DRACAENA LOUREIRI* STEM

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ด้วยความพยายามที่จะหาข้อมูลสนับสนุนการใช้จันทน์แดง (*Dracaena loureiri*) สำหรับรักษาอาการปวดและการอักเสบ ได้ทำการค้นหาสารจากแก่นของต้นไม้นี้และสามารถแยกสารบริสุทธิ์ได้ 9 ชนิด ประกอบด้วย retrodihydrochalcone 4 ชนิด, homoisoflavanone 2 ชนิด และ stilbene 3 ชนิด โครงสร้างของสารประกอบที่แยกได้นี้อธิบายและแปรผลได้จากข้อมูลทางสเปคโตรสโคปี ได้แก่ UV, IR, MS และ NMR พบว่าเป็นสารบริสุทธิ์ชนิดใหม่ 1 ชนิด คือ 3,5,7-trihydroxy-3-(4-hydroxybenzyl)-4-chromanone ซึ่งเป็นสารในกลุ่ม homoisoflavanone สำหรับสารบริสุทธิ์ที่แยกได้อีก 8 ชนิดเป็นสารที่มีการรายงานมาก่อนแล้ว ประกอบด้วย 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone, 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone, loureirin B, loureirin D, 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone, pterostilbene, pinostilbene และ resveratrol ได้ทำการทดสอบฤทธิ์ในการยับยั้งเอนไซม์ไซโคลออกซิเจนเนส-2 ของสารบริสุทธิ์แต่ละชนิด พบว่า pterostilbene, pinostilbene และ resveratrol มีฤทธิ์แรงที่สุด จากข้อมูลเหล่านี้แสดงว่าสารในกลุ่ม stilbenoid เป็นสารกลุ่มที่มีความสำคัญในการออกฤทธิ์ ซึ่งเป็นสิ่งสนับสนุนข้อบ่งใช้ทางยาของ *D.loureiri*

ภาควิชา เภสัชเวท	ลายมือชื่อนิสิต.....
สาขาวิชา เภสัชเวท	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา 2544	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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In an attempt to find supporting evidence for the reputed analgesic and antiinflammatory properties of *Dracaena loureiri*, a chemical investigation of the stem of this plant was initiated, and this led to the isolation of nine pure compounds, including four retrodihydrochalcones, two homoisoflavanones and three stilbenes. Through interpretation of their spectroscopic data (UV, IR, MS and NMR), these isolates were identified as a new homoisoflavanone, the structure of which was assigned as 3,5,7-trihydroxy-3-(4-hydroxybenzyl)-4-chromanone, and eight known compounds, including 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone, 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone, loureirin B, loureirin D, 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone, pterostilbene, pinostilbene and resveratrol. Each of these compounds was evaluated for its COX-2 inhibitory activity. It was found that pterostilbene, pinostilbene and resveratrol possessed the most potent activity. The data suggested that these stilbenoids could be the active principles responsible for the medicinal claims of *D. loureiri*.

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Academic year 2001 — Co-advisor's signature

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LIST OF ABBREVIATIONS

acetone-d_6	=	Deuterated acetone
Anti-PGE_2	=	Prostaglandin-E_2 antibody
BMMC	=	Bone marrow derived mast cells
BSA	=	Bovine serum albumin
br	=	Broad (for NMR spectra)
°C	=	Degree Celcius
CD_3OD	=	Deuterated methanol
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
cm	=	Centimeter
^{13}C NMR	=	Carbon-13 nuclear magnetic resonance
COLOC	=	Correlation spectroscopy via long-rang coupling
COSY	=	Correlation spectroscopy
COX-1	=	Cyclooxygenase-1
COX-$1^{-/-}$	=	Cyclooxygenase-1 null cell
COX-2	=	Cyclooxygenase-2
COX-$2^{-/-}$	=	Cyclooxygenase-2 null cell
cpm	=	Count per minute
CPMA	=	Count per minute average
1-D	=	One dimentional
2-D	=	Two dimentional
d	=	doublet (for NMR spectra)
dd	=	doublet of doublet (for NMR spectra)

DEPT	=	Distortionless enhancement by polarization transfer
DHA	=	Docosahexaenoic acid
DMEM	=	Dulbecco's modified Eagle medium
DMSO-d_6	=	Deuterated dimethylsulfoxide
δ	=	Chemical shift
EIMS	=	Electron Impact Mass Spectrum
EPA	=	Eicosapentaenoic acid
EtOAc	=	Ethyl acetate
g	=	Gram
Glc*p*	=	Glucopyranoside
HETCOR	=	Heteronuclear chemical shift correlation
^{1}H NMR	=	Proton nuclear magnetic resonance
HMBC	=	^{1}H detected heteronuclear multiple bond correlation
^{3}H-PGE$_2$	=	Tritium prostaglandin-E$_2$
Hz	=	Hertz
IC_{50}	=	Median inhibitory concentration
IR	=	Infrared spectrum
J	=	Coupling constant
KBr	=	Potassium bromide
KH_2PO_4	=	Potassium biphosphate
K_2HPO_4	=	Potassium phosphate
kg	=	Kilogram
L	=	Liter
α-LNA	=	α-Linolenic acid
LPS	=	Lipopolysaccharide

μg	=	Microgram
μl	=	Microliter
μM	=	Micromolar
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
M^+	=	Molecular ion
m	=	Multiplet (for NMR spectra)
mg	=	Milligram
MeOH	=	Methanol
MHz	=	Megahertz
ml	=	Milliliter
m/z	=	Mass to charge ratio
MS	=	Mass spectrometry
NaCl	=	Sodium chloride
$NaHCO_3$	=	Sodium bicarbonate
NaOH	=	Sodium hydroxide
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NOESY	=	Nuclear Overhauser effect correlation spectroscopy
PGE_2	=	Prostaglandin-E_2
PMA	=	Phorbol 12-myristate 13-acetate
ppm	=	Part per million
ν_{max}	=	Wave number at maximal absorption
q	=	Quartet (for NMR spectra)
Rha*p*	=	Rhamnopyranoside

RIA	=	Radioimmuno assay
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
TLC	=	Thin layer chromatography
TPA	=	12-*O*-Tetradecanoylphorbol 13-acetate
UV	=	Ultraviolet
Xyl*p*	=	Xylopyranoside

CHAPTER I

INTRODUCTION

Cyclooxygenase (COX), also known as prostaglandin H (PGH) synthase, catalyzes the first committed step in arachidonic acid metabolism. Two isoforms of the membrane protein COX are known: COX-1, which is constitutively expressed in most tissues, is responsible for the physiological production of prostaglandins; and COX-2, which is induced by cytokines, mitogens and endotoxins in inflammatory cells, is responsible for the elevated production of prostaglandins during inflammation (Kurumbail *et al*., 1996). COX-2 induces fever, pain and inflammation. Selective inhibitors of COX-2 should provide good candidates for anti-inflammatory drugs, having no effect on the expression of constitutive COX-1 (Seibert *et al*., 1994). In Thai traditional medicine, several medicinal plants have been used as antipyretics, including *Dracaena loureiri* Gagnep. (Saralamp *et al*., 1996).

The genus *Dracaena* belongs to the family Dracaenaceae of the order Liliales. This genus consists of some 100 species native to tropical regions such as Hawaii, Central America, Cuba and Macaronesia. They have also been introduced to the other tropical parts of the world (Bos, 1998).

Plants of the genus *Dracaena* are sometimes tree-like, but mostly are shrubby with single or few stems; leaves are various, either long and sword-shaped or broad and more or less distinctly petioled, often marked with stripes, bands or dots, in mature plants they are usually crowded at summit of trunk or branches; flowers are many, mostly in little clusters or fascicles arranged in panicles, small and not showy, greenish, whitish, yellowish; perianth is funnelform or narrowly bell-shaped, with long or short tube, the 6 segments are nearly or quite alike and spreading or reflexed;

stamens are 6, inserted on tube or throat, filaments are various, anthers are versatile. Ovary is nearly or quite sessile, 3-celled, style slender, stigma is capitate or somewhat lobed; fruits are globose berries, with 1-3 seeds (Bailey, 1949).

According to Smitinand (2001), eleven species of genus *Dracaena* have been identified in Thailand:

Dracaena angustifolia Roxb.	ค้อนหมาขาว Khon ma khao (Central), ผักก้อนหมา Phak khon ma (Lampang), พร้าวพันลำ Phrao phan lam (Chiang Mai), อีกริมป่า I krim pa (Chon Buri)
D. conferta Ridl. (*D. robusta* Ridl.)	กำลังขุนมาร Kamlang khunman (Nakorn Si Thammarat), กำลังควายถึก Kamlang khwai thuek (Yala), กำลังหนุมาน Kamlang hanuman (Peninsular), สะลีกี่บูโต๊ะ Sa-li-ki-bu-to (Malay- Narathiwat)
D. draco (L.) L.	เลือดมังกร Luet mangkon (Central), Dragon's Blood
D. elliptica Thunb.	หมากผู้ป่า Mak phu pa (Chiang Rai)
D. fragrans (L.) Ker Gawl.	ต้นวาสนา ton wassana, ประเดหวี Pra dewi (Bangkok)
D. granulata Hook.f.	หมากผู้ป่า Mak phu pa (Narathiwat), เหนียวสัง Niao sang (Narathiwat)
D. loureiri Gagnep.	จันทน์แดง Chan daeng (Central, Surat Thani), จันทน์ผา Chan pha (Northern), ลักกะจันทน์ Lakka chan (Central)
D. pachyphylla Kurz.	หมากผู้ป่า Mak phu pa (Chiang Rai)
D. pendula Ridl.	ข้าวเหนียวหมูสัง Khao niao mu sang (Pattani)
D. tenuiflora Roxb.	หมากผู้ป่า Mak phu pa (Chiang Rai)

D. umbratica Ridl. เกาะนาซิ Ko-na-si (Malay-Narathiwat)

Dracaena loureiri Gagnep.is an indigenous plant known in Thai as Chan daeng or Lakkachan. It is a shrub, up to 4 m high; the leaves are simple, alternate, crowded at the top, linear, 5-7 cm wide, 50-70 cm long; the inflorescence is in terminal, large panicle, bent downward; the flowers are small, yellowish white; fruits are globose baccates. The dried stem from *D. loureiri* Gagnep. has been used as an antipyretic in Thai traditional medicine (Saralamp *et al*., 1996).

To date, no study has been done regarding the anti-inflammatory activity of this plant. In our preliminary evaluation of the COX-2 inhibitory activity of *D. loureiri*, an ethyl acetate extract of this plant showed potent inhibition (97 % inhibition at 100 μg/ml). The main objectives in this investigation are as follows.

1. Isolation and purification of constituents of the stem of *D. loureiri.*
2. Determination of the chemical structure of each isolated compound.
3. Evaluation of the COX-2 inhibitory potential of each isolated compound.

Figure 1 *Dracaena loureiri* Gagnep. (Saralamp et al., 1996)

CHAPTER II

HISTORICAL

1. Chemical Constituents of *Dracaena*

A number of compounds have been isolated from the genus *Dracaena*. They can be classified as flavonoids, steroids, steroidal saponins, triterpenoids, stilbenes, benzenoids, alkanes, lipids and inorganic compounds as shown in Table 1.

Table 1 Distribution of chemical constituents in the genus *Dracaena.*

Plant and chemical compound	Category	Plant part	References
Dracaena afromontana			
Afromontoside [**1**]	steroid	twig	Reddy *et al*., 1984
Diosgenin [**2**]	steroid	twig	Reddy *et al*., 1984
D. cambodiana			
Dioscin [**3**]	steroidal saponin	fruit	Yang and Wang, 1986
22-Methylprotodioscin [**4**]	steroidal saponin	fruit	Yang and Wang, 1986
Gracillin [**5**]	steroidal saponin	fruit	Yang and Wang, 1986
22-Methylgracillin [**6**]	steroidal saponin	fruit	Yang and Wang, 1986
D. cinnabari			
7-Hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman [**7**]	flavonoid	resin	Masaoud *et al*., 1995c
7-Hydroxy-3-(4-hydroxy-benzyl)-8-methoxychroman [**8**]	flavonoid	resin	Masaoud *et al*., 1995c

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. cinnabari			
3-(4-Hydroxybenzyl)-7,8-methylenedioxychroman [**9**]	flavonoid	resin	Masaoud *et al*., 1995c
7-Hydroxy-3-(4-hydroxy benzyl)chroman [**10**]	flavonoid	resin	Masaoud *et al*., 1995c
(±)-7,4′-Dihydroxy-3-methoxy flavan [**11**]	flavonoid	resin	Masaoud *et al*., 1995c
(2*S*)-7,3′-Dihydroxy-4′-methoxyflavan [**12**]	flavonoid	resin	Masaoud *et al*., 1995c
(2*S*)-7-Hydroxyflavan [**13**]	flavonoid	resin	Masaoud *et al*., 1995c
4-Hydroxy-2-methoxydihydro-chalcone [**14**]	flavonoid	resin	Masaoud *et al*., 1995c
4,4′-Dihydroxy-2-methoxy-dihydrochalcone (Loureirin C) [**15**]	flavonoid	resin	Masaoud *et al*., 1995c
4,4′-Dihydroxy-2′-methoxy chalcone [**16**]	flavonoid	resin	Masaoud *et al*., 1995c
7,4′-Dihydroxyflavone [**17**]	flavonoid	resin	Masaoud *et al*., 1995c
(2*S*)-7-Hydroxyflavanone [**18**]	flavonoid	resin	Masaoud *et al*., 1995c
2′-Methoxysocotrin-5′-ol [**19**]	flavonoid	resin	Masaoud *et al*., 1995a
Socotrin-4′-ol [**20**]	flavonoid	resin	Masaoud *et al*., 1995a

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. cinnabari			
Homoisosocotrin-4′-ol [**21**]	flavonoid	resin	Masaoud *et al*., 1995a
Cinnabarone [**22**]	flavonoid	resin	Masaoud *et al*., 1995b
Damalachawin [**23**]	flavonoid	resin	Himmelreich *et al*., 1995
Betulin [**24**]	triterpenoid	root	Masaoud, Schmidt and Adam, 1995d
Lophenol [**25**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
4α,14α-Dimethylcholest-8-en-3β-ol [**26**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
Cycloartanol [**27**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
24-Methylenecycloartanol [**28**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
31-Norcycloartanol [**29**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
Lupeol [**30**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
Cholest-4-en-3-one [**31**]	steroid	resin	Masaoud, Schmidt and Adam, 1995d

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. cinnabari			
Cholesterol [**32**]	steroid	root	Masaoud, Schmidt and Adam, 1995d
Stigmasterol [**33**]	steroid	root	Masaoud, Schmidt and Adam, 1995d
Campesterol [**34**]	steroid	root	Masaoud, Schmidt and Adam, 1995d
Lanost-7-en-3β-ol [**35**]	triterpenoid	resin	Masaoud, Schmidt and Adam, 1995d
Sitosterol [**36**]	steroid	root	Masaoud, Schmidt and Adam, 1995d
Stigmastanol [**37**]	steroid	root	Masaoud, Schmidt and Adam, 1995d
Stigmast-22-en-3β-ol [**38**]	steroid	root	Masaoud, Schmidt and Adam, 1995d
3-(4-Hydroxybenzyl)-7,8-methylenedioxychroman [**9**]	flavonoid	not specified	Juranek *et al*., 1993
7-Hydroxy-3-(4-hydroxy-benzyl)-8-methoxychroman [**8**]	flavonoid	not specified	Juranek *et al*., 1993
7-Hydroxy-3-(4-hydroxy-benzyl)chroman [**10**]	flavonoid	not specified	Juranek *et al*., 1993

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. cochinchinensis			
1,2,4,5-Tetrachloro-3,6-dimethoxybenzene [**39**]	benzenoid	not specified	Tang *et al*.,1995
Docosan-1-ol acetate [**40**]	alkane	not specified	Tang *et al*.,1995
Eicosan-1-ol acetate [**41**]	alkane	not specified	Tang *et al*.,1995
Octadecane-1-ol acetate [**42**]	alkane	not specified	Tang *et al*.,1995
Resveratrol [**43**]	stilbene	not specified	Tang *et al*.,1995
Docosanyl ferulate [**44**]	alkane	not specified	Wei *et al*., 1998
7,3′-Dihydroxy-4′-methoxy-flavone [**45**]	flavonoid	not specified	Wei *et al*., 1998
n-Heptacosane [**46**]	alkane	not specified	Wei *et al*., 1998
Hexacosanyl ferulate [**47**]	alkane	not specified	Wei *et al*., 1998
Lophenol [**25**]	triterpenoid	not specified	Wei *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. cochinchinensis			
Octacosanyl ferulate [**48**]	Alkane	not specified	Wei *et al*., 1998
Dioctyl phathalate [**49**]	benzenoid	not specified	Wei *et al*., 1998
Butylisobutyl phathalate [**50**]	benzenoid	not specified	Wei *et al*., 1998
Pterostilbene [**51**]	stilbene	not specified	Wei *et al*., 1998
		bark	Lu *et al*., 1998
Tetracosanyl ferulate [**52**]	alkane	not specified	Wei *et al*., 1998
4′-Hydroxy-2,4,6-trimethoxy-dihydrochalcone (Loureirin B) [**53**]	flavonoid	bark	Lu *et al*., 1998
4′-Hydroxy-2,6-dimethoxy-dihydrochalcone (Loureirin A) [**54**]	flavonoid	bark	Lu *et al*., 1998
6-Hydroxy-7-methoxy-3-(4-hydroxybenzyl)chroman [**55**]	flavonoid	bark	Lu *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. cochinchinensis			
7-Hydroxy-4′-methoxyflavan [**56**]	flavonoid	bark	Lu *et al*., 1998
		resin	Wang *et al*., 1995
		stem	Wang *et al*., 1999b
4-Hydroxybenzoic acid methyl ester [**57**]	benzenoid	resin	Wang *et al*., 1995
7,4′-Dihydroxyflavan [**58**]	flavonoid	resin	Wang *et al*., 1995
		stem	Wang *et al*., 1999b
7,4′-Dihydroxyflavone [**17**]	flavonoid	resin	Wang *et al*., 1995
D. concinna			
(25*R*)-Spirost-5-en-3*β*-ol (diosgenin) 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside} [**59**]	steroidal saponin	leaves	Mimaki *et al*., 1998
Diosgenin 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside} [**60**]	steroidal saponin	leaves	Mimaki *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. concinna			
(25*R*)-Spirost-5-ene-1*β*,3*β*-diol (ruscogenin) 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyraside}[**61**]	steroidal saponin	leaves	Mimaki *et al*., 1998
Ruscogenin 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-sulfo-*α*-L-arabinopyranoside} [**62**]	steroidal saponin	leaves	Mimaki *et al*., 1998
(23*S*,24*S*,25*S*)-Spirost-5-ene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside} [**63**]	steroidal saponin	leaves	Mimaki *et al*., 1998
(23*S*,24*S*,25*S*)-Spirost-5-ene-1*β*,3*β*, 23,24-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside} 24-*O*-*β*-D-fucopyranoside [**64**]	steroidal saponin	leaves	Mimaki *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. concinna			
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl -(25*R*)-furost-5-ene-3*β*,22*ξ*,26-triol 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside} [**65**]	steroidal saponin	leaves	Mimaki *et al*., 1998
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl -(25*R*)-furost-5-ene-3*β*,22*ξ*,26-triol 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside} [**66**]	steroidal saponin	leaves	Mimaki *et al*., 1998
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1*β*,3*β*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-fucopyranoside [**67**]	steroidal saponin	leaves	Mimaki *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. concinna			
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*α*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside} [**68**]	steroidal saponin	leaves	Mimaki *et al*., 1998
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*α*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside} [**69**]	steroidal saponin	leaves	Mimaki *et al*., 1998
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*α*,4*α*,22*ξ*, 26-pentol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside} [**70**]	steroidal saponin	leaves	Mimaki *et al*., 1998

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. concinna			
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*β*,4*α*,22*ξ*,26-pentol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside} [**71**]	steroidal saponin	leaves	Mimaki *et al*., 1998
Concinnasteoside A [**72**]	steroidal saponin	stem	Mimaki *et al*., 1997
D. draco			
(25*R*)-Spirost-5-en-3*β*-ol 3-*O*-{*O*-*α*-L- rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside} [**73**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999
Spirost-5,25(27)-diene-1*β*,3*β*-diol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside} [**74**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. draco			
(23*S*)-Spirosta-5,25(27)-diene-1*β*,3*β*,23-triol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside} [**75**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999
26-*O*-*β*-D-Glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1*β*,3*β*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside} [**76**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999
(23*S*, 24*S*)-Spirosta-5,25(27)-diene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-(2,3,4-tri-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside} 24-*O*-*β*-D-fucopyranoside [**77**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. draco			
(23*S*, 24*S*)-Spirosta-5,25(27)-diene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyrano-side} [**78**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999
(23*S*, 24*S*)-Spirosta-5,25(27)-diene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-(4-*O*- acetyl-*α*-L-rhamno-pyranosyl)-(1→2)-*α*-L-arabinopyranoside} [**79**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999
(23*S*)-Spirosta-5,25(27)-diene-1*β*,3*β*,23-triol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside} [**80**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999
(23*S*)-Spirosta-5,25(27)-diene-1*β*,3*β*,23-triol 1-*O*-{*O*-(4-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1→2)-*α*-L-arabinopyrano-side} [**81**]	steroidal saponin	aerial parts	Mimaki *et al*., 1999

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. draco			
7,4′-Dihydroxyhomoiso-flavanone [**82**]	flavonoid	resin	Camarda *et al*., 1983
5,7-Dihydroxy-3-(4-hydroxy-benzyl)-4-chromanone [**83**]	flavonoid	resin	Camarda *et al*., 1983
5,7,4′-Trihydroxy-6-methyl-homoisoflavanone [**84**]	flavonoid	resin	Camarda *et al*., 1983
7-Hydroxy-3-(4-hydroxy-benzyl)chroman [**10**]	flavonoid	resin	Camarda *et al*., 1983
7-Hydroxy-3-(4-hydroxy-benzyl)-8-methoxychroman [**8**]	flavonoid	resin	Camarda *et al*., 1983
(2*S*)-7,4′-Dihydroxy-3′-methoxy-8-methylflavan [**85**]	flavonoid	resin	Camarda *et al*., 1983
(2*S*)-5,4′-Dihydroxy-7-methoxy-8-methylflavan [**86**]	flavonoid	resin	Camarda *et al*., 1983
3,4′-Dihydroxy-7-methoxy-flavone [**87**]	flavonoid	resin	Camarda *et al*., 1983
2,4,4′-Trihydroxydihydro-chalcone [**88**]	flavonoid	resin	Gonzalez *et al*., 2000
3-(4-Hydroxybenzyl)-5,7-dimethoxychroman [**89**]	flavonoid	resin	Gonzalez *et al*., 2000

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. draco			
Diosgenin [**2**]	steroid	bark	Gonzalez *et al*., 1972
Dracogenin [**90**]	steroid	bark	Gonzalez *et al*., 1972
Neoruscogenin [**91**]	steroid	bark	Gonzalez *et al*., 1972
D. loureiri			
10-Hydroxy-11-methoxy-dracaenone [**92**]	flavonoid	stem	Meksuriyen *et al*., 1987
7,10-Dihydroxy-11-methoxy-dracaenone [**93**]	flavonoid	stem	Meksuriyen *et al*., 1987
7-Hydroxy-3-(4-hydroxy-benzyl)chroman [**10**]	flavonoid	stem	Meksuriyen and Cordell, 1988a
Loureirin A [**54**]	flavonoid	stem	Meksuriyen and Cordell, 1988a
Loureirin B [**53**]	flavonoid	stem	Meksuriyen and Cordell, 1988a
Loureirin C [**15**]	flavonoid	stem	Meksuriyen and Cordell, 1988a
Loureirin D [**94**]	flavonoid	stem	Meksuriyen and Cordell, 1988a
7,4′-Dihydroxyflavone [**17**]	flavonoid	stem	Meksuriyen and Cordell, 1988b

Table 1 (continued)

Plant and chemical compound	Category	Plant part	References
D. loureiri			
(2*S*)-7-Hydroxyflavanone [**18**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
(2*S*)-Pinocembrin [**95**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
(2*S*)-7,4′-Dihydroxy-5-methoxyflavanone [**96**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
4,4′-Dihydroxy-2′-methoxy-chalcone [**16**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
(2*R*)-7,4′-Dihydroxyflavan [**97**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
(2*R*)-4′-Hydroxy-7-methoy-flavan [**98**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
(3*R*)-Eucomol [**99**]	flavonoid	stem	Meksuriyen and Cordell, 1988b
4,4′-Dihydroxy-2,6-dimethoxy dihydrochalcone [**100**]	flavonoid	stem	Ichikawa *et al*., 1997
2,4′-Dihydroxy-4,6-dihydroxy dihydrochalcone [**101**]	flavonoid	stem	Ichikawa *et al*., 1997
5,7-Dihydroxy-3-(4-hydroxy-benzyl)-4-chromanone [**83**]	flavonoid	stem	Ichikawa *et al*., 1997

Table 1 (continued)

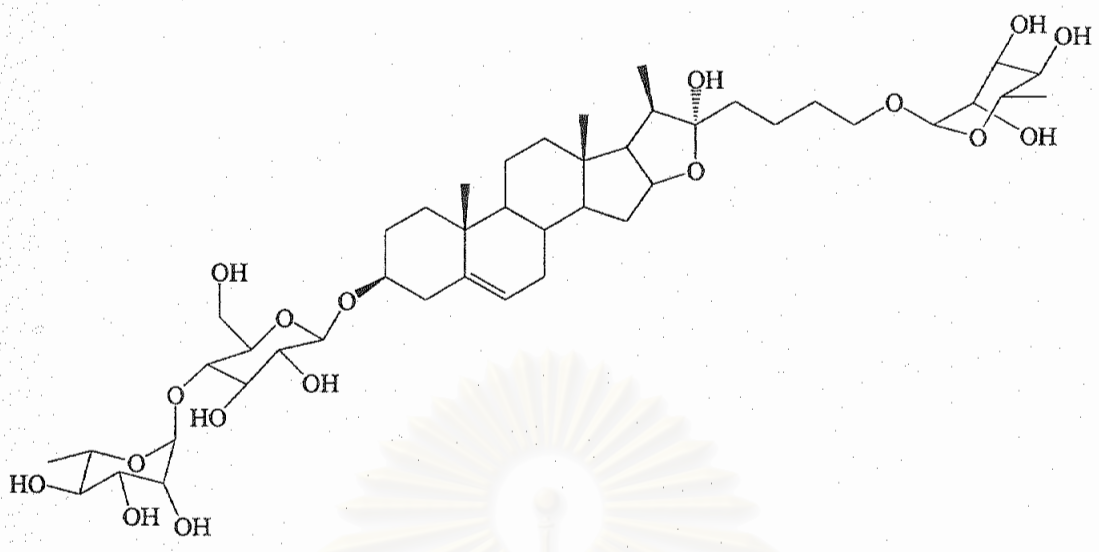
Plant and chemical compound	Category	Plant part	References
D. loureiri			
7-Hydroxy-3-(4-hydroxy benzyl)-5-methoxy-4-chromanone [**102**]	flavonoid	stem	Ichikawa *et al*., 1997
D. mannii			
3β-*O*-[(α-L-rhamnopyranosyl-(1→2), α-L-rhamnopyranosyl-(1→3))-β-D-glucopyranosyl]-17α-hydroxyl-spirost-5-ene (DM-1) [**103**]	steroidal saponin	fruit pulp	Okunji *et al*., 1990
Palmitic acid [**104**]	lipid	stem bark	Sofowora and Olaniyi, 1975
Ammonium nitrate [**105**]	inorganic	stem bark	Sofowora and Olaniyi, 1975
n-Heptacosane [**47**]	alkane	stem bark	Sofowora and Olaniyi, 1975
D. niten			
Diosgenin [**2**]	steroid	root bark	Bila and Tandu, 1988
D. reflexa			
Capric acid [**106**]	lipid	seeds	Daulatabad *et al*., 1982
Lauric acid [**107**]	lipid	seeds	Daulatabad *et al*., 1982
Myristic acid [**108**]	lipid	seeds	Daulatabad *et al*., 1982

Table 1 (continued)

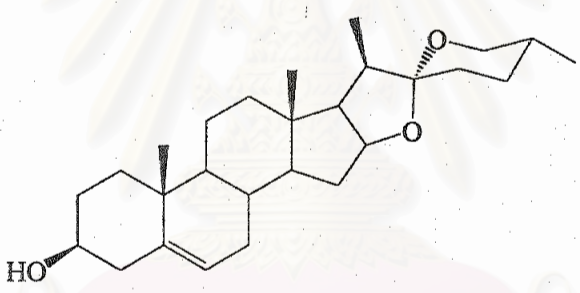
Plant and chemical compound	Category	Plant part	References
D. reflexa			
Palmitic acid [**104**]	lipid	seeds	Daulatabad *et al*., 1982
Steric acid [**109**]	lipid	seeds	Daulatabad *et al*., 1982
Arachidic acid [**110**]	lipid	seeds	Daulatabad *et al*., 1982
Oleic acid [**111**]	lipid	seeds	Daulatabad *et al*., 1982
Linoleic acid [**112**]	lipid	seeds	Daulatabad *et al*., 1982
D. surculosa			
Surculoside A [**113**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000
Serculoside B [**114**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000
Serculoside C [**115**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000
(25*S*)-1β-[(β-D-Glucopyranosyl)oxy]-3β-hydroxy-22α-methoxyfurost-5-en-26-yl β-D-glucopyranoside [**116**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000
(25*R*)-17α-Hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranoside [**117**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000

Table 1 (continued)

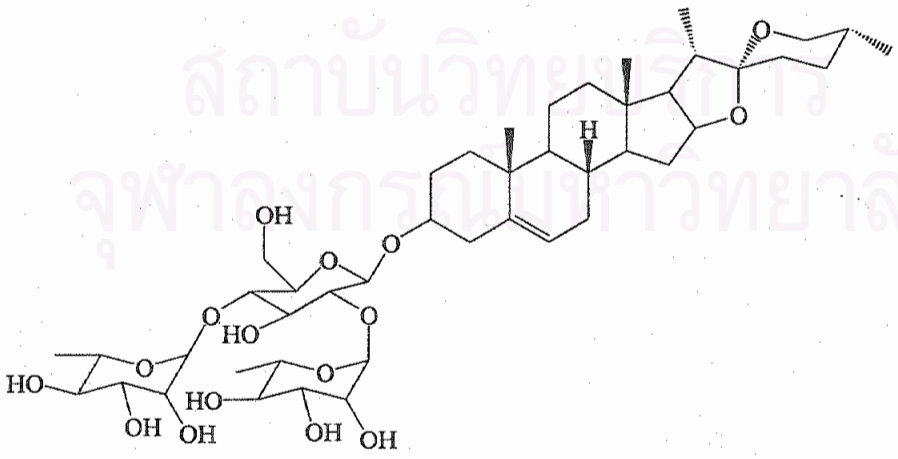
Plant and chemical compound	Category	Plant part	References
D. surculosa			
(25*R*)-17α-Hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1$\rightarrow$2)- *O*-[α-L-rhamnopyranosyl-(1$\rightarrow$4)]-β-D-glucopyranoside [**118**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000
(25*S*)-3β-Hydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-fucopyranoside [**119]**	steroidal saponin	whole plant	Yokosuka *et al*., 2000
(25*S*)-1β-[(β-D-Fucopyranosyl)oxy] 3β-hydroxy-22α-methoxyfurost-5-en-26-yl β-D-glucopyranoside [**120**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000
(25*S*)-3β-Hydroxy-22α-methoxy-1β-[(2-*O*-α-L-rhamnopyranosyl-β-D-fucopyranosyl)oxy]furost-5-en-26-yl β-D-glucopyranoside [**121**]	steroidal saponin	whole plant	Yokosuka *et al*., 2000



[1] Afromontoside

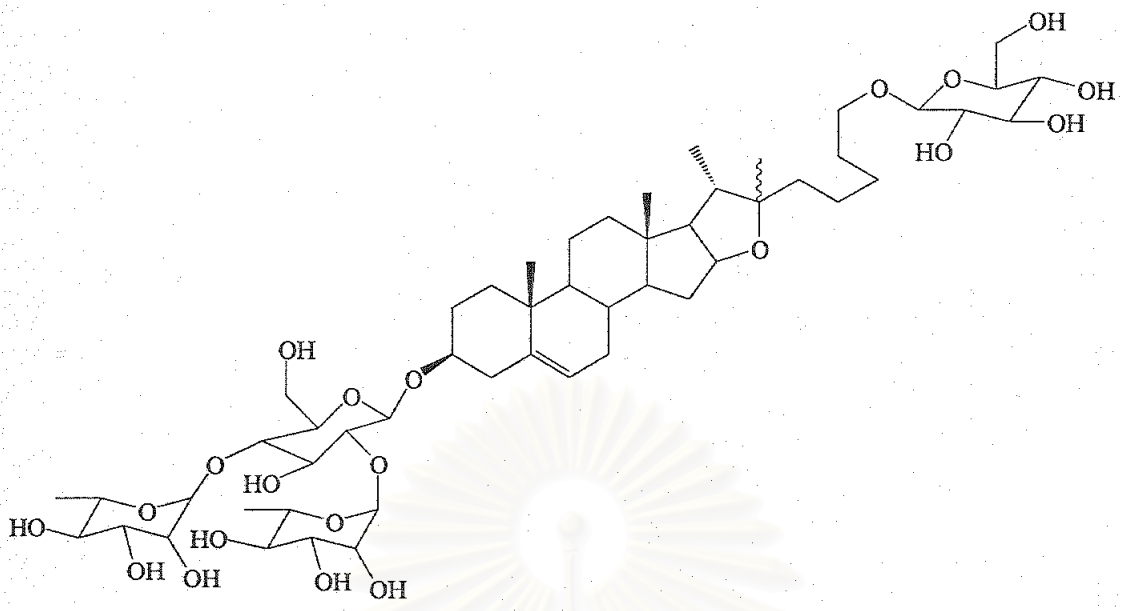


[2] Diosgenin

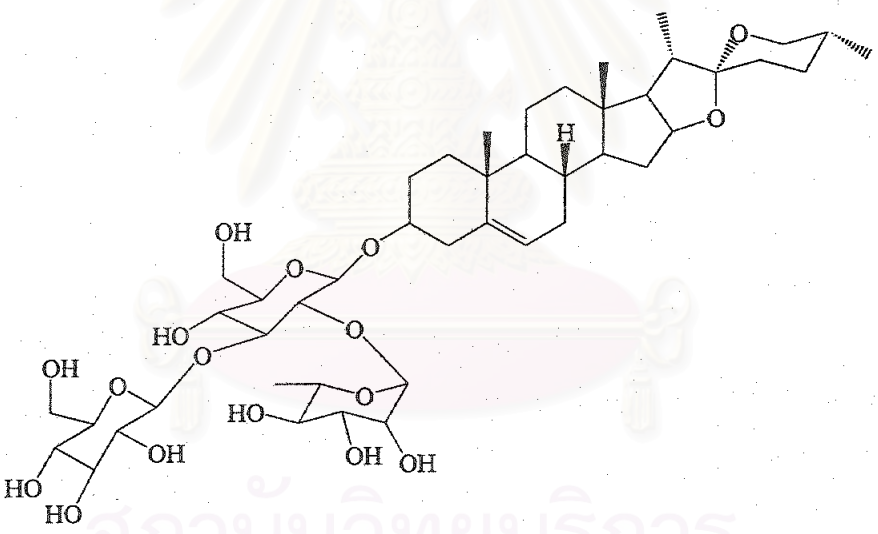


[3] Dioscin

Figure 2 Structures of compounds previously isolated from the *Dracaena*

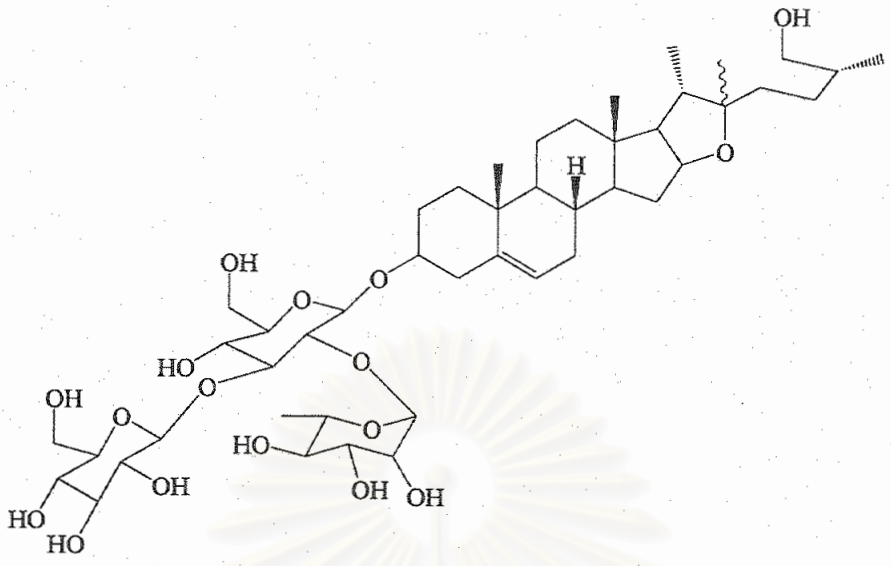


[4] 22-Methylprotodioscin

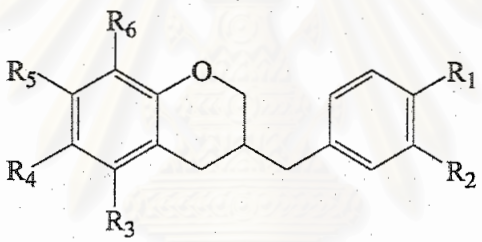


[5] Gracillin

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

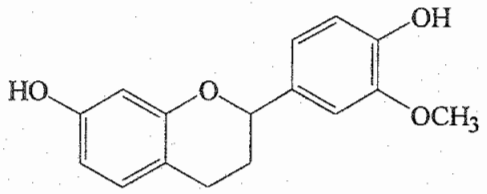


[6] 22-Methylgracillin

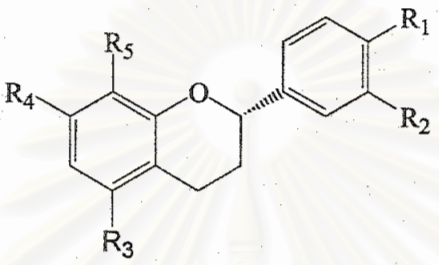


R_1	R_2	R_3	R_4	R_5	R_6	
OCH_3	OH	H	H	OH	H	[7] 7-Hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman
OH	H	H	H	OH	OCH_3	[8] 7-Hydroxy-3-(4-hydroxy-benzyl)-8-methoxychroman
OH	H	H	H	O-CH_2-O		[9] 3-(4-Hydroxybenzyl)-7,8-methylenedioxychroman
OH	H	H	H	OH	H	[10] 7-Hydroxy-3-(4-hydroxy-benzyl)chroman

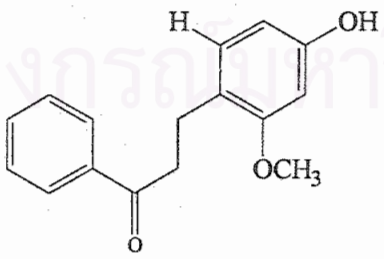
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



[**11**] (±)-7,4′-Dihydroxy-3′-methoxyflavan

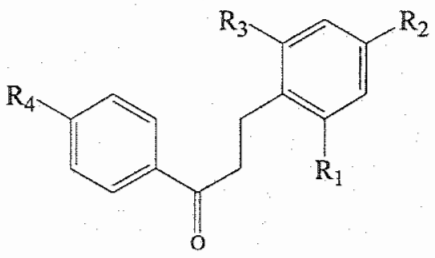


R_1	R_2	R_3	R_4	R_5	
OH	OCH_3	H	OH	H	[**12**] (2*S*)-7,3′-Dihydroxy-4′-methoxyflavan
H	H	H	OH	H	[**13**] (2*S*)-7-Hydroxyflavan
OH	OCH_3	H	OH	CH_3	[**85**] (2*S*)- 7,4′-Dihydroxy-3′-methoxy-8-methylflavan
OH	H	OH	OCH_3	CH_3	[**86**] (2*S*)-5,4′-Dihydroxy-7-methoxy-8-methylflavan

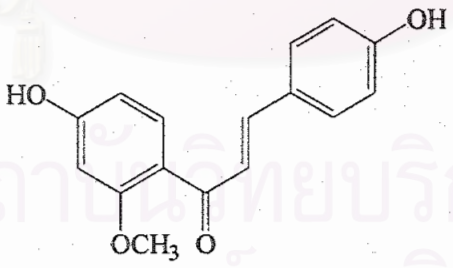


[**14**] 4-Hydroxy-2-methoxydihydrochalcone

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

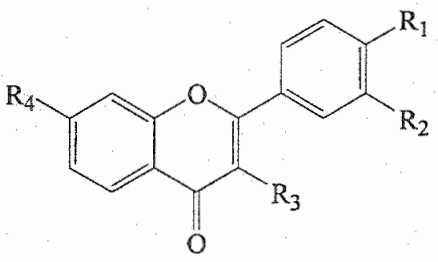


R_1	R_2	R_3	R_4	
OCH_3	OH	H	OH	[**15**] Loureirin C
OH	OH	H	OH	[**88**] 2,4,4′-Trihydroxydihydro-chalcone
OCH_3	OH	OH	OH	[**94**] Loureirin D
OCH_3	OH	OCH_3	OH	[**100**] 4,4′-Dihydroxy-2,6-dimethoxydihydrochalcone
OH	OCH_3	OCH_3	OH	[**101**] 2,4′-Dihydroxy-4,6-dimethoxydihydrochalcone

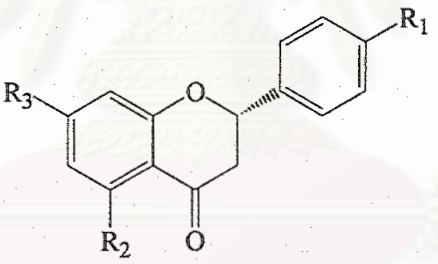


[**16**] 4,4′-Dihydroxy-2′-methoxychalcone

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

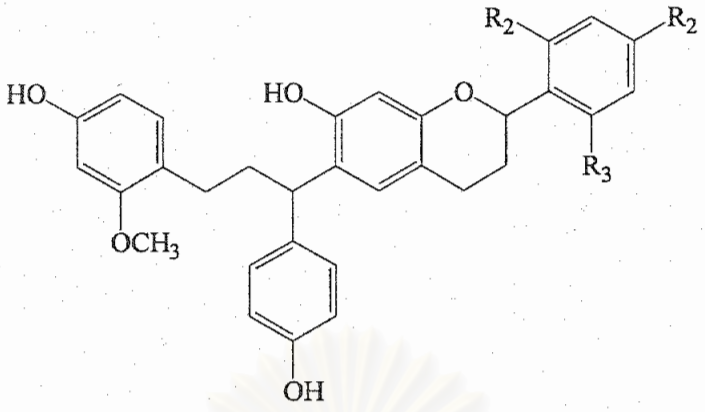


R_1	R_2	R_3	R_4	
OH	H	H	OH	[**17**] 7,4′-Dihydroxyflavone
OCH_3	OH	H	OH	[**45**] 7,3′-Dihydroxy-4′-methoxyflavone
OH	H	OH	OCH_3	[**87**] 3,4′-Dihydroxy-7-methoxyflavone

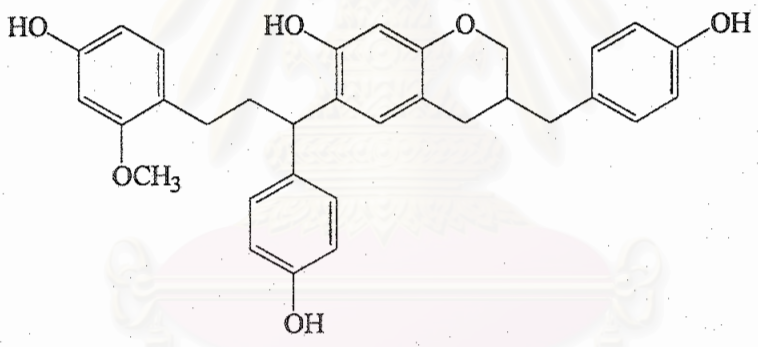


R_1	R_2	R_3	
H	H	OH	[**18**] (2*S*)-7-Hydroxyflavanone
H	OH	OH	[**95**] (2*S*)-Pinocembrin
OH	OCH_3	OH	[**96**] (2*S*)-7,4′-Dihydroxy-5-methoxyflavanone

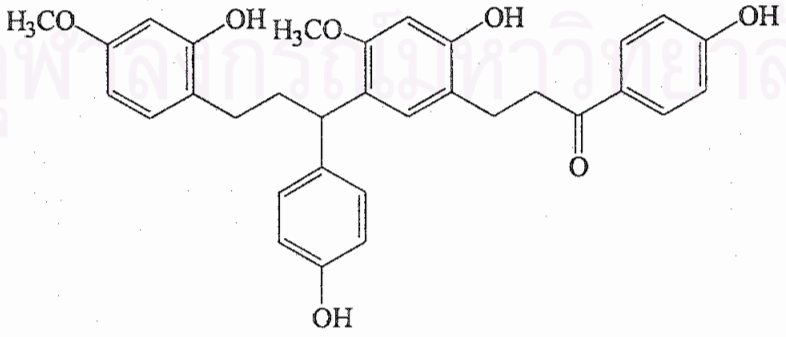
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



R_1	R_2	R_3	
OCH_3	H	OH	[19] 2′-Methoxysocotrin-5′-ol
H	OH	H	[20] Socotrin-4′-ol

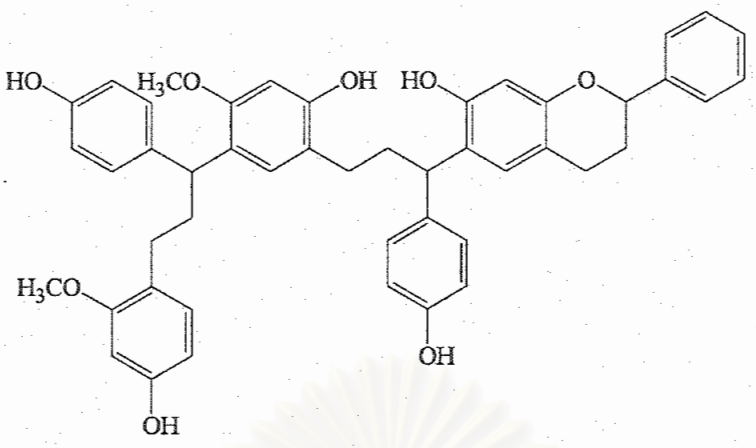


[21] Homoisosocotrin-4′-ol

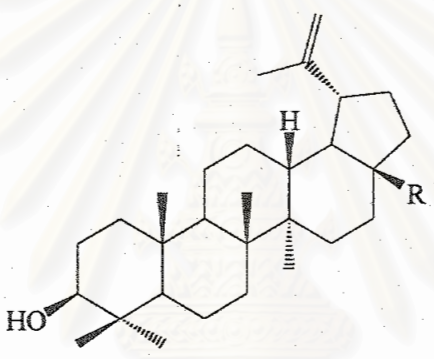


[22] Cinnabarone

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

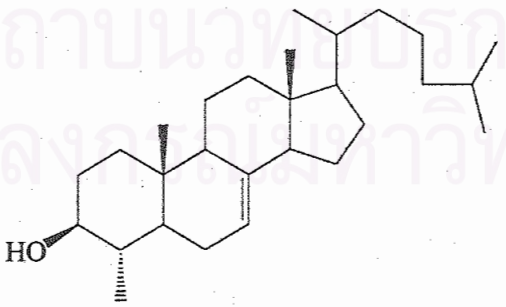


[23] Damalachawin



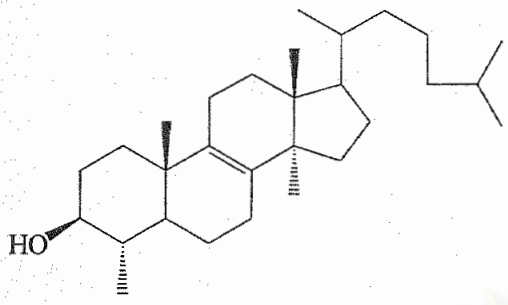
[24] Betulin R = CH_2OH

[30] Lupeol R = CH_3

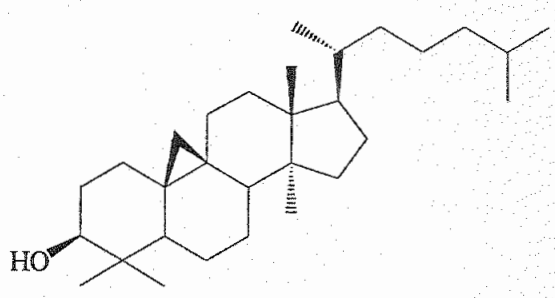


[25] Lophenol

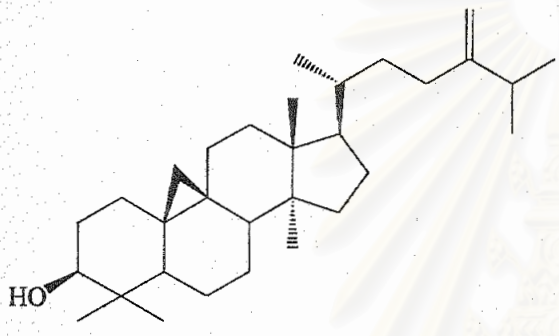
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



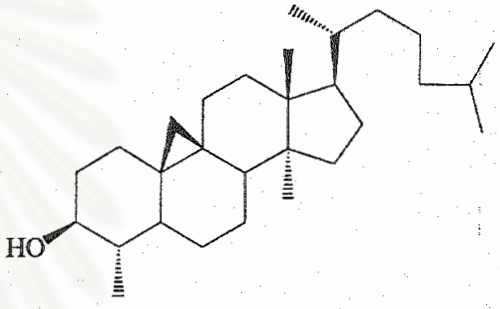
[**26**] 4α,14α-Dimethylcholest-8-en-3β-ol



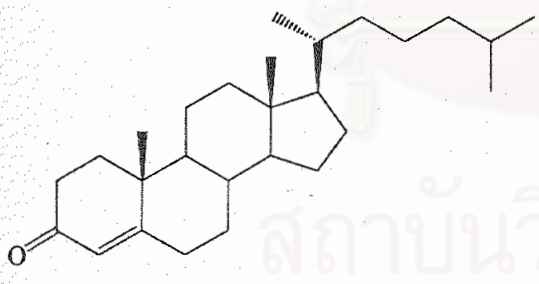
[**27**] Cycloartanol



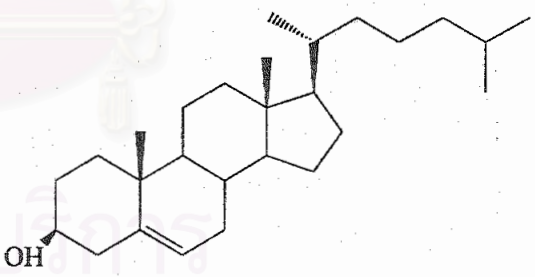
[**28**] 24-Methylenecycloartanol



[**29**] 31-Norcycloartanol

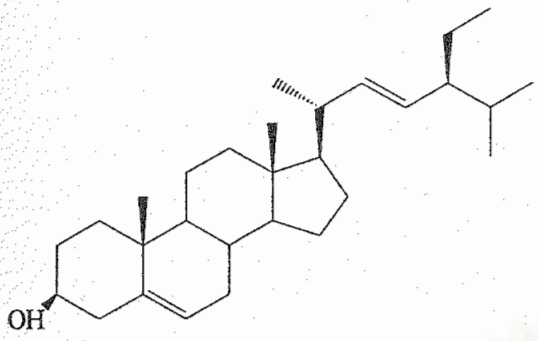


[**31**] Cholest-4-en-3-one

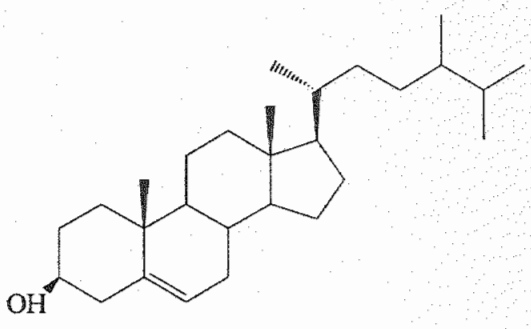


[**32**] Cholesterol

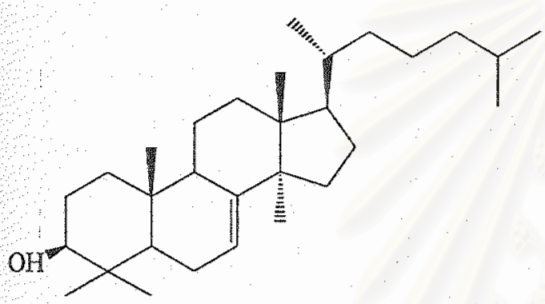
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



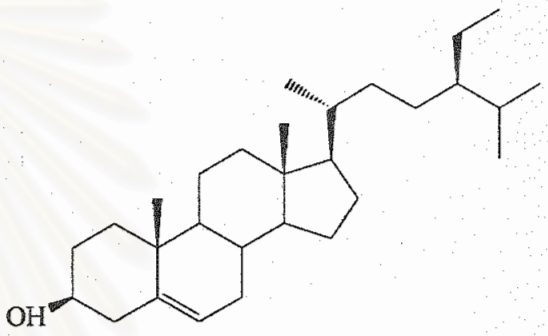
[**33**] Stigmasterol



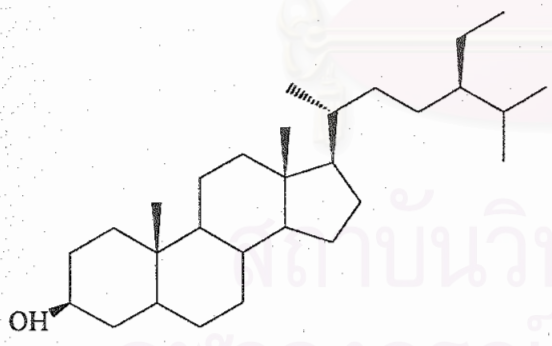
[**34**] Campesterol



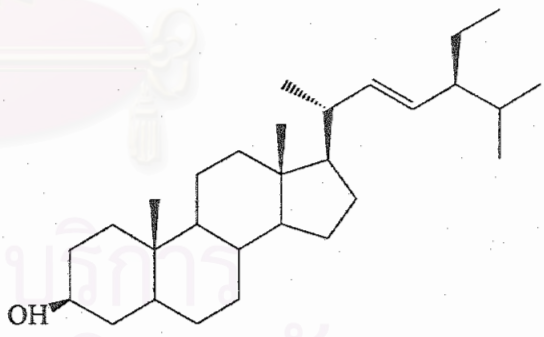
[**35**] Lanost-7-en-3β-ol



[**36**] sitosterol

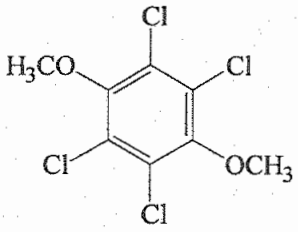


[**37**] Stigmastanol

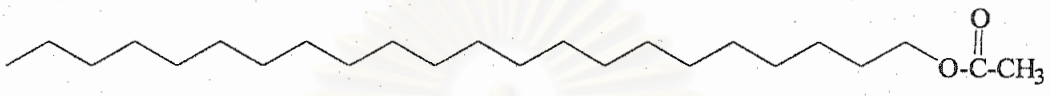


[**38**] Stigmast-22-en-3β-ol

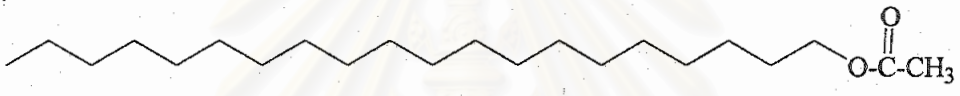
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



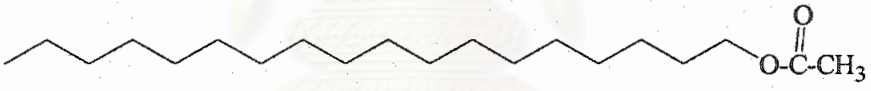
[**39**] 1,2,4,5-Tetrachloro-3,6-dimethoxybenzene



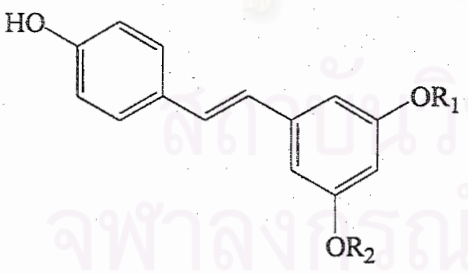
[**40**] Docosan-1-ol acetate



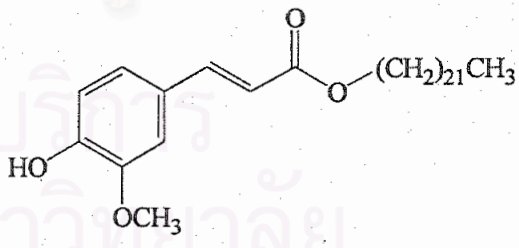
[**41**] Eicosan-1-ol acetate



[**42**] Octadecan-1-ol acetate

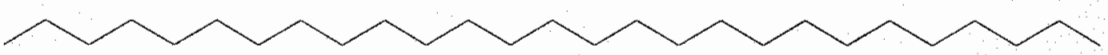


R_1	R_2	
H	H	[**43**] Resveratrol
CH_3	CH_3	[**51**] Pterostilbene

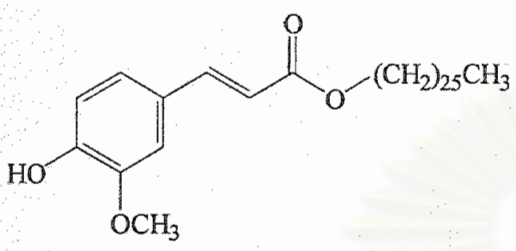


[**44**] Docosanyl ferulate

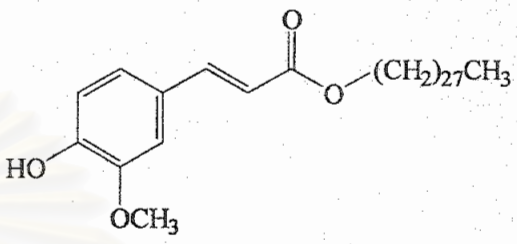
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



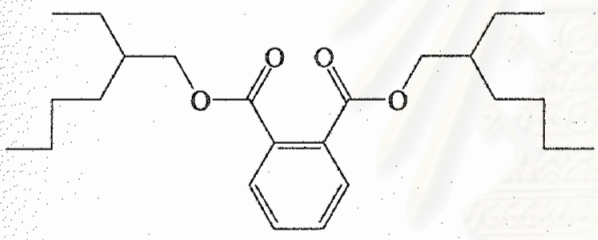
[46] n-Heptacosane



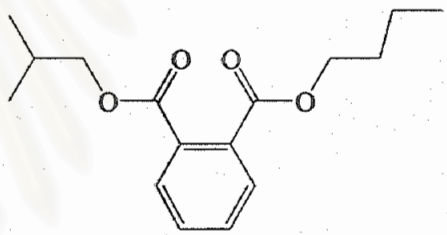
[47] Hexacosanyl ferulate



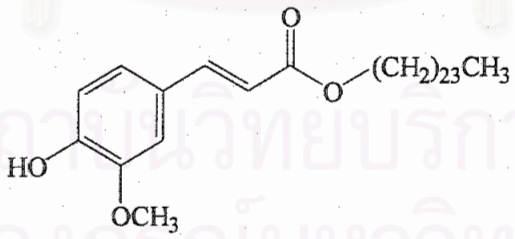
[48] Octacosanyl ferulate



[49] Dioctyl phathalate

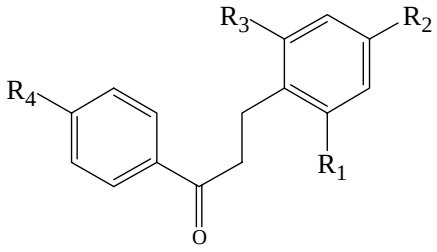


[50] Butylisobutyl phathalate

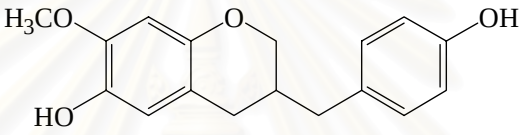


[52] Tetracosanyl ferulate

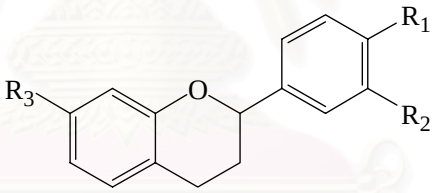
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



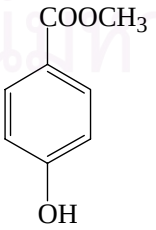
R_1	R_2	R_3	R_4	
OCH_3	OCH_3	OCH_3	OH	[**53**] Loureirin B
OCH_3	OCH_3	H	OH	[**54**] Loureirin A



[**55**] 6-Hydroxy-7-methoxy-3-(4-hydroxybenzyl)chroman

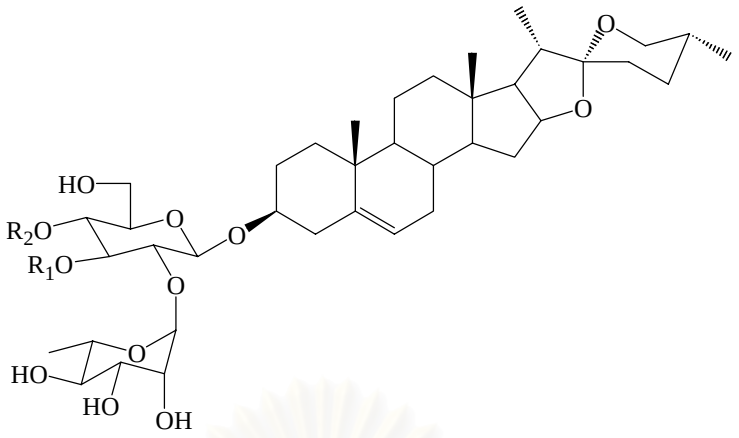


R_1	R_2	R_3	
OCH_3	H	OH	[**56**] 7-Hydroxy-4′-methoxyflavan
OH	H	OH	[**58**] 7,4′-Dihydroxyflavan

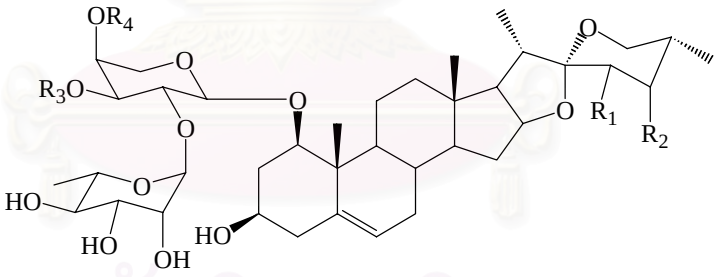


[**57**] 4-Hydroxybenzoic acid methyl ester

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

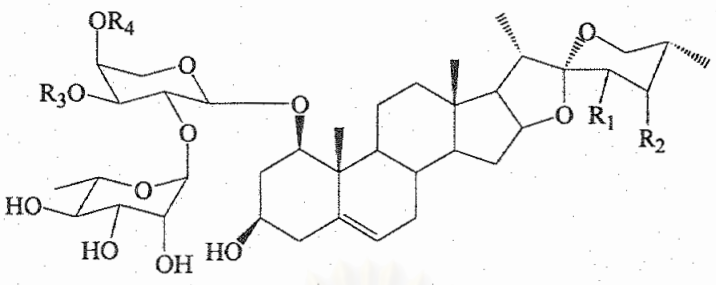


R_1	R_2	
H	α-L-Rha*p*	[**59**] (25*R*)-Spirost-5-en-3β-ol (diosgenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside}
β-D-Glc*p*	H	[**60**] Diosgenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside}



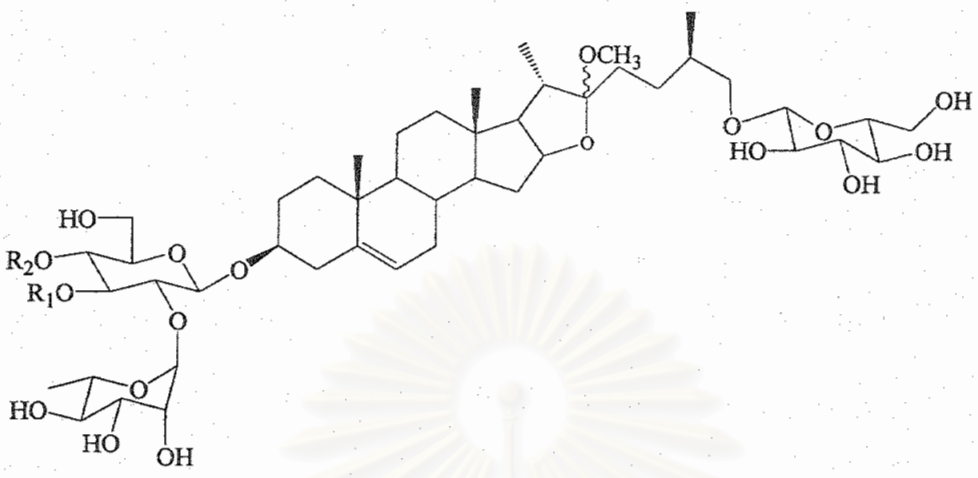
R_1	R_2	R_3	R_4	
H	H	H	H	[**61**] (25*R*)-Spirost-5-en-1β,3β-diol (ruscogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}
H	H	H	SO_3^-	[**62**] Ruscogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-4-*O*-sulfo-α-L-arabinopyranoside}

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



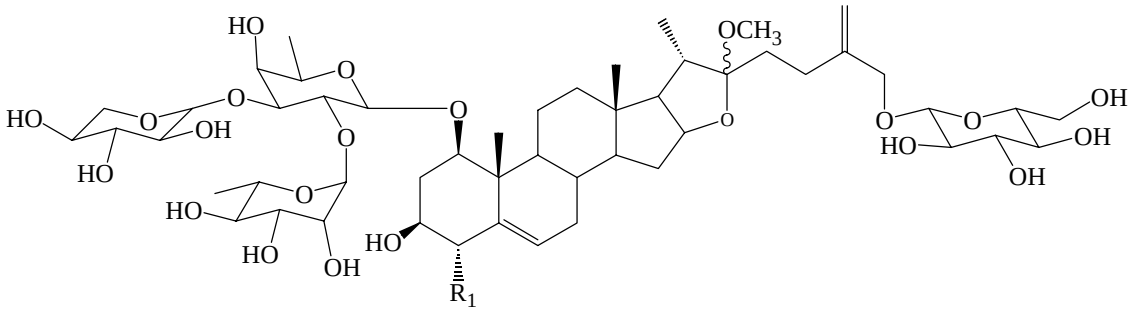
R_1	R_2	R_3	R_4		
OH	OH	*β*-D-Xyl*p*	H	**[63]**	(23*S*,24*S*,25*S*)-Spirost-5-ene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-*α*-L-rhamnopyra nosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside}
OH	*O*-*β*-D-fuc*p*	*β*-D-Xyl*p*	H	**[64]**	(23*S*,24*S*,25*S*)-Spirost-5-ene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside} 24-*O*-*β*-D-fucopyranoside

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

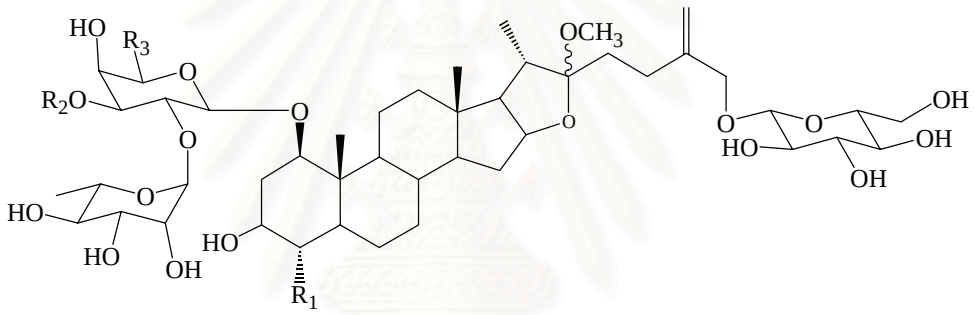


R_1	R_2	
H	*α*-L-Rha*p*	[**65**] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-(25*R*)-furost-5-ene-3*β*,22*ξ*,26-triol 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)])-*β*-D-glucopyranoside}
β-D-Glc*p*	H	[**66**] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-(25*R*)-furost-5-ene-3*β*,22*ξ*,26-triol 3-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)-*β*-D-gluco-pyranoside}

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

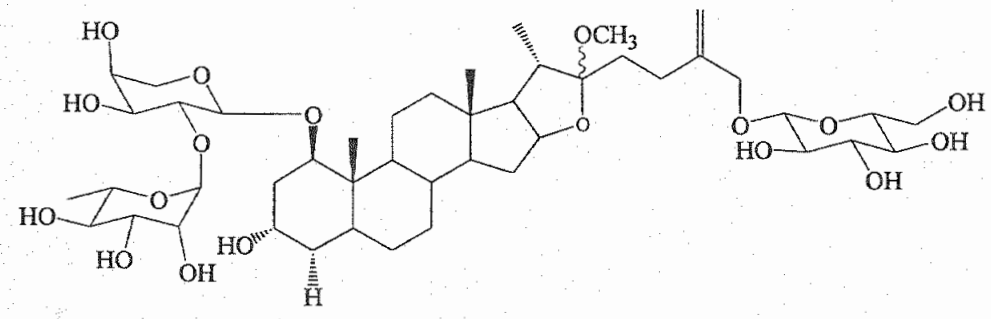


[**67**] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1*β*,3*β*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-fucopyranoside}

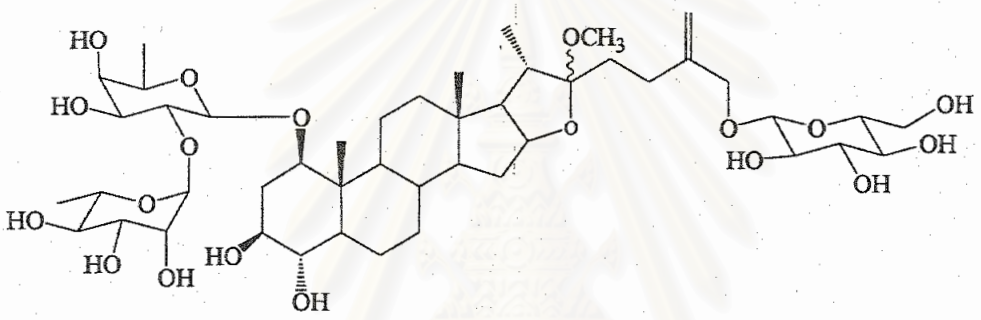


R_1	R_2	R_3	
H	H	CH_3 (3*α*)	[**68**] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*α*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside}
OH	H	CH_3 (3*α*)	[**70**] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*α*,4*α*,22*ξ*,26-pentol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside}

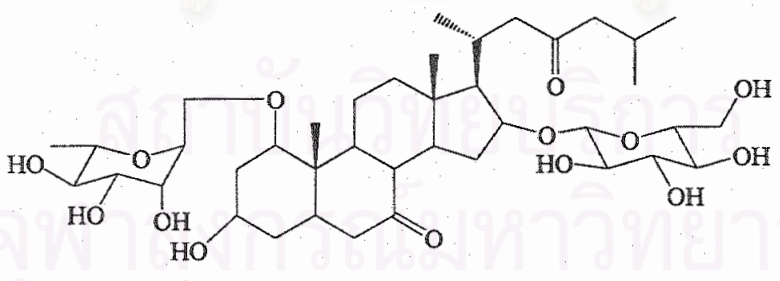
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



[69] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*α*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside}

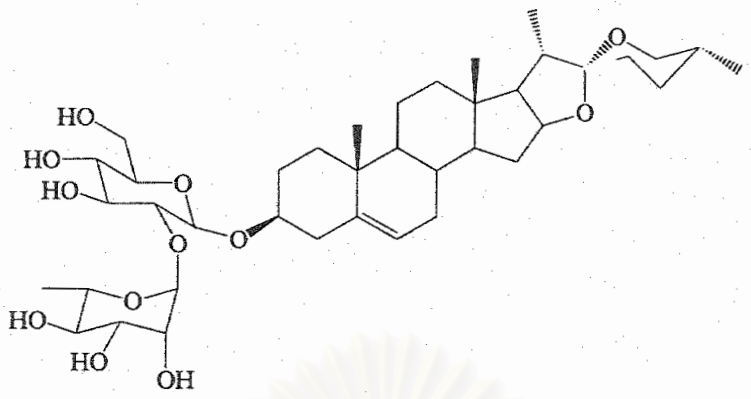


[71] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methyl-5*α*-furost-25(27)-ene-1*β*,3*β*,4*α*,22*ξ*,26-pentol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside}

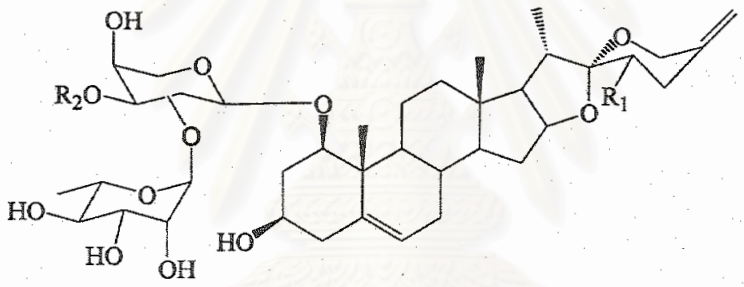


[72] Concinnasteoside A

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

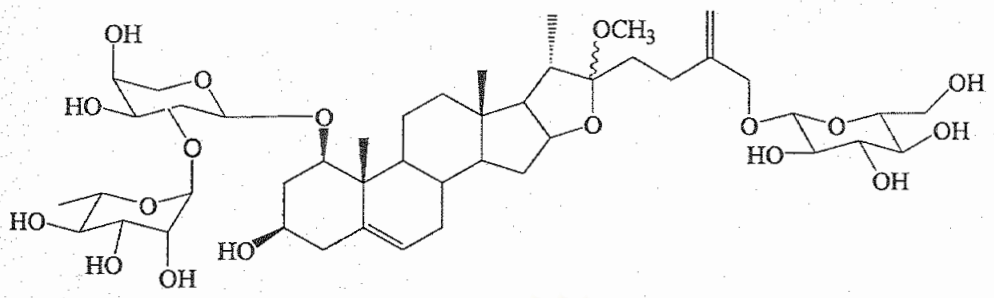


[73] (25*R*)-Spirost-5-en-3β-ol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-gluco-pyranoside}

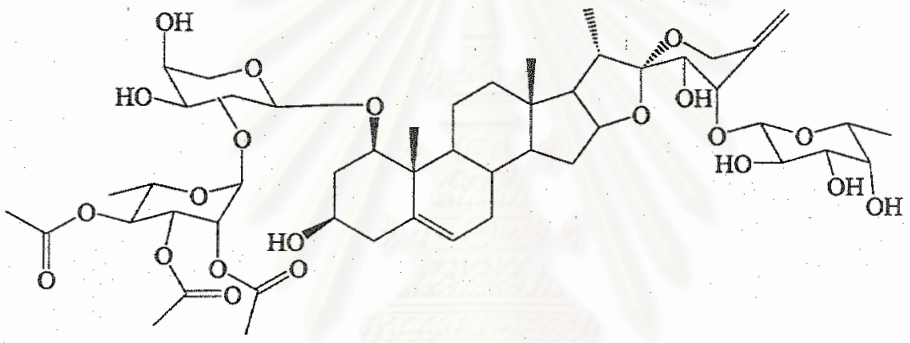


R_1	R_2	
H	H	[74] Spirost-5,25(27)-diene-1β,3β-diol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}
OH	β-D-Xyl*p*	[75] (23*S*)-Spirost-5,25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylo-pyranosyl-(1→3)]-α-L-arabinopyranoside}

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

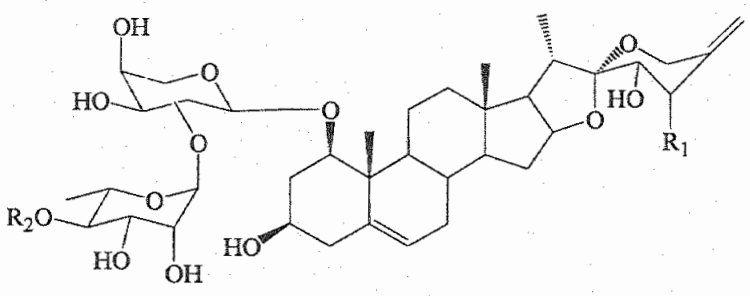


[76] 26-*O*-*β*-D-Glucopyranosyl-22-*O*-methylfurosta-5, 25(27)-diene-1*β*,3*β*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside}

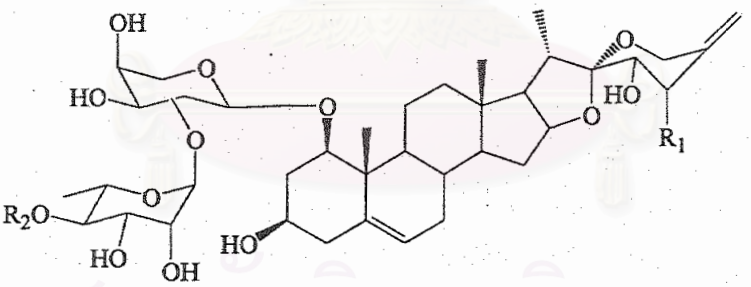


[77] (23*S*,24*S*)-Spirost-5, 25(27)-diene-1*β*,3*β*,23,24-tetrol 1-*O*-{*O*-(2,3,4-tri-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl} 24-*O*-*β*-D-fucopyranoside

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

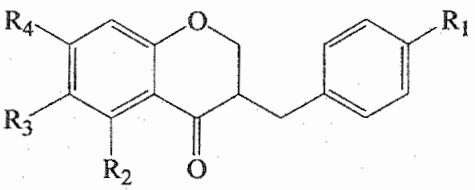


R_1	R_2	
OH	H	[78] (23*S*,24*S*)-Spirost-5, 25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabino-pyranoside}
OH	Ac	[79] (23*S*,24*S*)-Spirost-5, 25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}

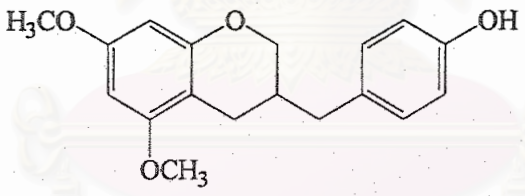


R_1	R_2	
H	H	[80] (23*S*)-Spirost-5, 25(27)-diene-1β, 3β, 23-triol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}
H	Ac	[81] (23*S*)-Spirost-5, 25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabino-pyranoside}

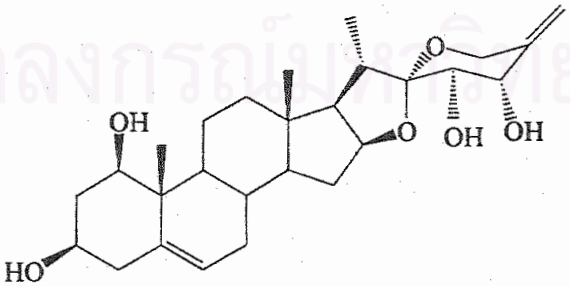
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



R_1	R_2	R_3	R_4	
OH	H	H	OH	[**82**] 7,4′-Dihydroxyhomoisoflavanone
OH	OH	H	OH	[**83**] 5,7-Dihydroxy-3-(4-hydroxybenzyl)-4-chromanone
OH	OH	CH_3	OH	[**84**] 5,7,4′-Trihydroxy-6-methylhomoiso-flavanone
OH	OCH_3	H	OH	[**102**] 7-Hydroxy-3-(4-hydroxybenzyl)5-methoxy-4-chromanone

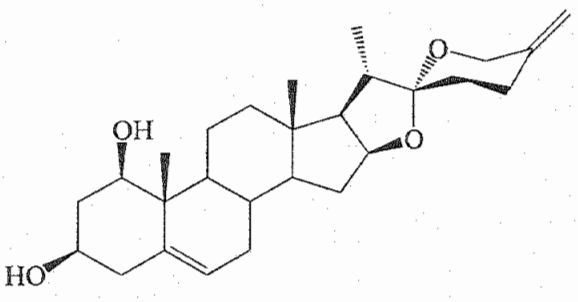


[**89**] 3-(4-Hydroxybenzyl)-5,7-dimethoxychroman

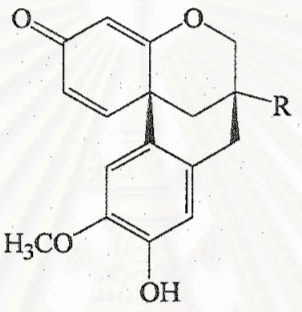


[**90**] Dracogenin

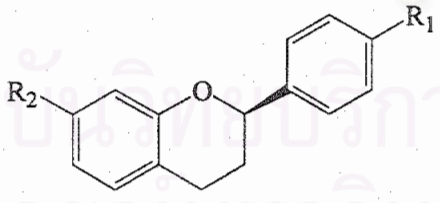
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



[**91**] Neoruscogenin

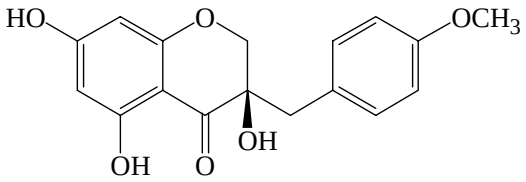


R	
H	[**92**] 10-Hydroxy-11-methoxydracaenone
OH	[**93**] 7,10-Dihydroxy-11-methoxydracaenone

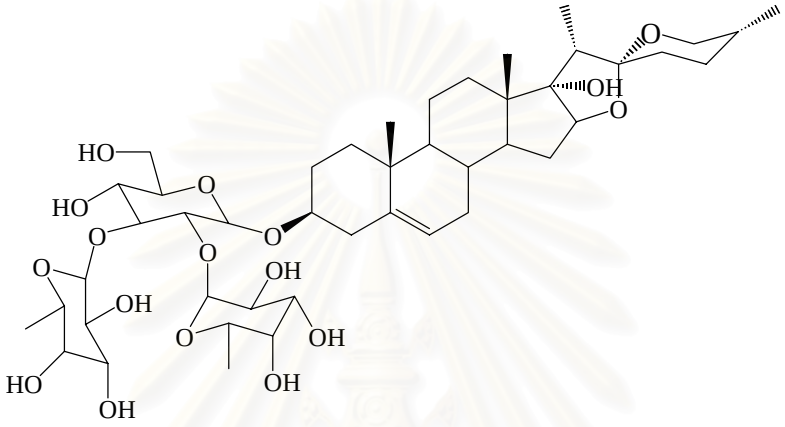


R_1	R_2	
OH	OH	[**97**] (2*R*)-7,4′-Dihydroxyflavan
OH	OCH_3	[**98**] (2*R*)-4′-Hydroxy-7-methoxyflavan

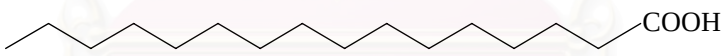
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



[**99**] 3*R*-Eucomol



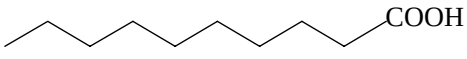
[**103**] 3β-*O*-[(α-L-rhamnopyranosyl-(1→2), α-L-rhamnopyranosyl-(1→3))-β-D-glucopyranosyl]-17α-hydroxyl-spirost-5-ene (DM-1)



[**104**] Palmitic acid

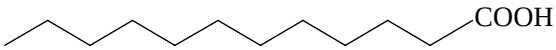
$NH_4^+NO_3^-$

[**105**] Ammonium nitrate

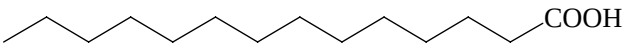


[**106**] Capric acid

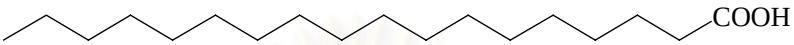
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



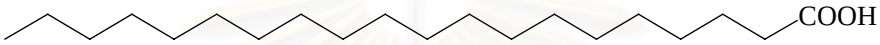
[**107**] Lauric acid



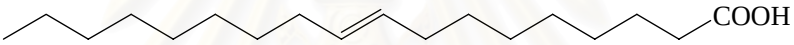
[**108**] Myristic acid



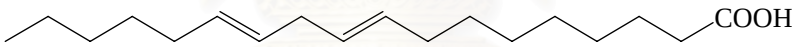
[**109**] Steric acid



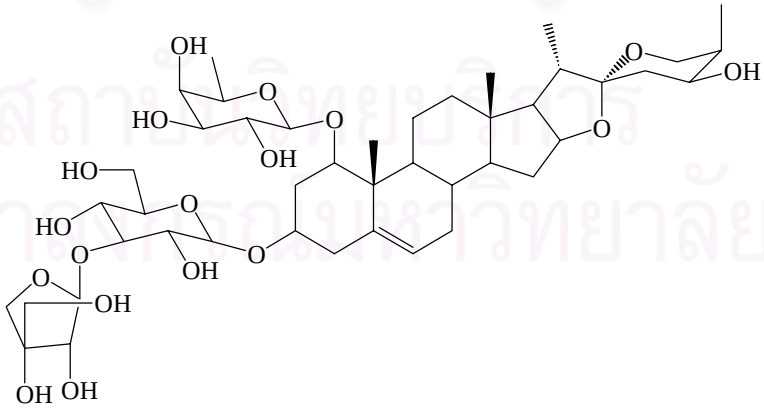
[**110**] Arachidic acid



[**111**] Oleic acid

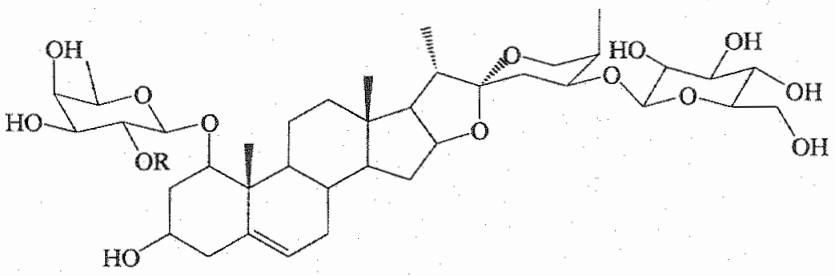


[**112**] Linoleic acid

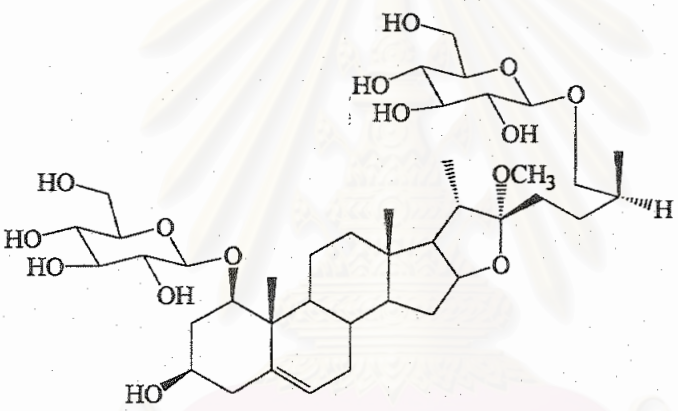


[**113**] Surculoside A

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

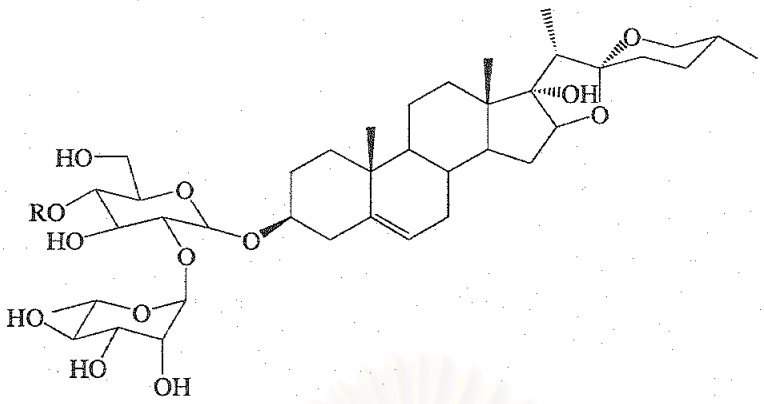


R	
H	[**114**] Surculoside B
α-L-Rha*p*	[**115**] Surculoside C



[**116**] (25*S*)-1*β*-[(*β*-D-Glucopyranosyl)oxy]-3*β*-hydroxy-22*α*-methoxyfurost-5-en-26-yl *β*-D-glucopyranoside

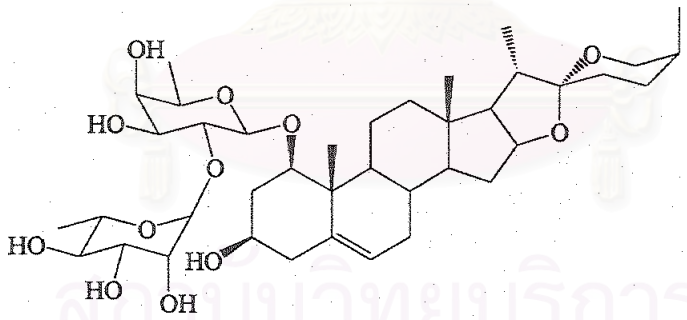
Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



R

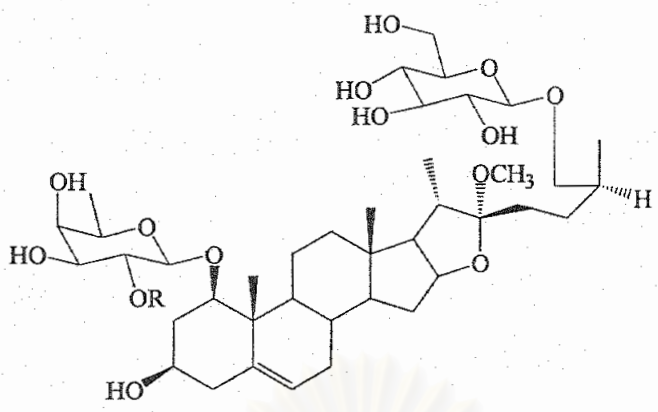
H [117] (25*R*)-17α-Hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside

β-D-Rha*p* [118] (25*R*)-17α-Hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside



[119] (25*S*)-3β-Hydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)



R	
H	**[120]** (25*S*)-1β-[(β-D-Fucopyranosyl)oxy]-3β-hydroxy-22α-methoxyfurost-5-en-26-yl β-D-glucopyranoside
α-L-Rha*p*	**[121]** (25*S*)-3β-Hydroxy-22α-methoxy-1β-[(2-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fuco-pyranosyl)oxy]furost-5-en-26-yl β-D-gluco-pyranoside

Figure 2 Structures of compounds previously isolated from the *Dracaena* (continued)

2. Traditional Uses and Biological Activities of *Dracaena* Constituents.

Plants of the genus *Dracaena* have been used medicinally in several countries: dried leaves of *D. afromontana* are used for the treatment of diarrhea in Rwanda (Maikere-Faniyo *et al*., 1989), and dried leaves and roots of this plant for the treatment of rheumatism in Kenya (Reddy *et al*., 1984); leaves and roots of *D. angustifolia* for the treatment of cold, malaria, rheumatism and kidney troubles in India (Maikhuri and Gangwar, 1993); dried resin of *D. cambodiana* to stop bleeding in China (Sheng-Ji, 1985); roots of *D. deremensis* for the treatment of hepatomegaly (Chhabra, Mahunnah and Mshiu, 1987), rheumatism, aphrodisiac, fever, malaria and to accelarated labor in Tanzania (Chhabra and Uiso, 1991); sap of *D. draco* as an anticarcinogen (Darias *et al*., 1986) and for the treatment of gingivitis in the Canary Islands (Darias *et al*., 1989); dried wood of *D. loureiri* as an antipyretic in Thailand (Saralamp *et al*., 1996); leaves of *D. reflexa* var. *angustifolia* for the treatment of skin problems in the Rodrigues Islands (Gurib-Fakim *et al*., 1996); and leaves and fruits of *D. steudneri* for the treatment of otitis, scabies, syphilis and yaws in Rwanda (Boily and Van Puyvelde, 1986; Vlietinch *et al*., 1995).

A number of biological investigations of *Dracaena* spp. have been reported. 4,4′-Dihydroxy-2′-methoxychalcone [**16**] from *D. loureiri* showed *in vitro* cytotoxic activity against KB cells (Meksuriyen and Cordell, 1988a), and several steroidal compounds from *D. surculosa* (Yokosuka *et al*., 2000) and *D. draco* (Mimaki *et al*., 1999) showed weak cytotoxic activity against leukemia HL-60 cells.

The flavonoids from the stem wood of *D. loureiri*, such as 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone [**100**] and 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**83**], displayed weak estrogen agonist activities (Ichikawa *et al*., 1997). Compound DM-1 [**103**] from the fruit pulp of *D. mannii* exhibited weak antifungal

(Okunji *et al*., 1990) and molluscicidal activities (Okunji, Iwu and Hostettmann, 1991). Some flavonoids from *D. loureiri*, such as loureirin D [**94**] and (2*S*)-pinocembrin [**95**], showed weak antibacterial activity againt *Bacillus subtilis* (Meksuriyen and Cordell, 1988b). The leaf extract of *D. reflexa* var. *nitens* inhibited the growth of *Escherichia paracoli*, *Citrobacter diversus* and *Pseudomonas aeruginosa*. In addition, this extract showed antiamoebic activity against *Entamoeba histolytica* and exerted weak antispasmodic activity on the ileum of guinea-pig (Tona *et al*., 1999).

3. COX-2 Inhibitors from Natural Sources.

Several groups of natural products have been shown to possess COX-2 inhibitory activitiy. They can be classified as:

3.1 Saponins

Platycodin D [**122**], from the root of *Platycodon grandiflorum* (Campanulaceae), was a saponin which inhibited 12-*O*-tetradecanoyl- phorbol 13-acetate (TPA)-induced PGE_2 production in rat peritoneal macrophages by inhibiting the induction of COX-2 protein, but did not directly inhibit COX-1, COX-2 and phospholipase A_2 at IC_{50} of 10 μM (Kim *et al*., 2001).

3.2 Stilbenes

Resveratrol [**43**] found in grapes exhibited dose-dependent suppression of phorbol ester (PMA)-induced COX-2 activity in 184B5/HER cells and inhibited recombinant human COX-2 enzyme activity at IC_{50} of 32.2 μM, which was less potent than the synthetic inhibitors NS-398 (IC_{50} = 3.2 μM) and indomethacin IC_{50} (IC_{50} = 1.9 μM) (Subbarramaiah *et al*., 1998). Aiphanol [**123**] and isorhapontigenin

[**124**], isolated from the seeds of *Aiphanes aculeata* (Araceae), showed weak COX-2 inhibitory activity (Lee *et al*., 2001).

3.3 Diarylheptanoids

Derivatives from the family Zingiberaceae, such as curcumins I-III [**125**-**127**] from the rhizomes of *Curcuma longa*, showed COX-2 inhibitory activity. Curcumin I [**125**] exhibited potent and selective COX-2 inhibitory activity (Ramsewak, De Witt and Nair, 2000), whereas yakuchinone B [**128**] and demethyl-yakuchinone B [**129**] from *Alpinia oxyphylla* showed weak COX-2 inhibitory effect (Yamazaki *et al*., 1998).

3.4 Quinazoline alkaloids

Tryptanthrin [**130**] from the leaves of *Isatis tingtoria* (Brassicaceae) exhibited potent (IC_{50} = 64 nM) COX-2 inhibitory activity in Mono Mac 6 cells stimulated by lipopolysaccharide (LPS), comparable with that of synthetic COX-2 inhibitors such as nimesulide (IC_{50} = 39 nM) and NS-398 (IC_{50} = 2 nM) (Danz *et al*., 2001). Rutaecarpine [**131**], from the fruits of *Evodia rutaecarpa* (Rutaceae), showed potent (IC_{50} = 0.28 μM) and selective COX-2 inhibitory activity in bone marrow derived mast cells (BMMC) (COX-1 IC_{50}/ COX-2 IC_{50} = 30) and in COX-2 tranfected HEX 293 cells (COX-1 IC_{50}/ COX-2 IC_{50} >140) (Moon *et al.*, 1999).

3.5 Terpenes

Pathenolide [**132**] from the leaves of *Magnolia grandiflora* (Magnoliaceae) showed COX-2 inhibitory activity in LPS-stimulated macrophage at IC_{50} of 0.8 μM (Hwang *et al*., 1996). Akendo 3 [**133**] from *Endospermum diadenum* (Euphorbiaceae) inhibited COX-2 more than 43% at 100 μM when tested on COX-2 catalyzed PGE_2 synthesis in LPS-stimulated microsomes from human leukocytes (Paya *et al*., 1996). Ursolic acid [**134**] from *Plantago major* (Plantaginaceae)

exhibited potent COX-2 inhibition (IC_{50} = 130 μM), as compared with NS-398 (IC_{50} = 53 μM) (Ringbom *et al*., 1998).

3.6 Fatty acids

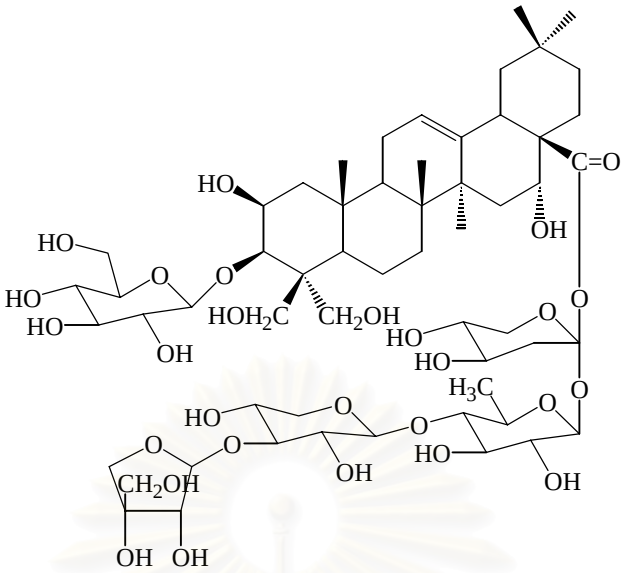
EPA [**135**] and DHA [**136**] from the cold water fishes, and *α*-LNA [**137**] from *P. major* showed COX-2 inhibitory activity at IC_{50} values of 7.1, 9.8 and 12 μM, respectively. They were more potent than NS-398 (IC_{50} = 53 μM) (Ringbom *et al*., 2001).

3.7 Flavonoids

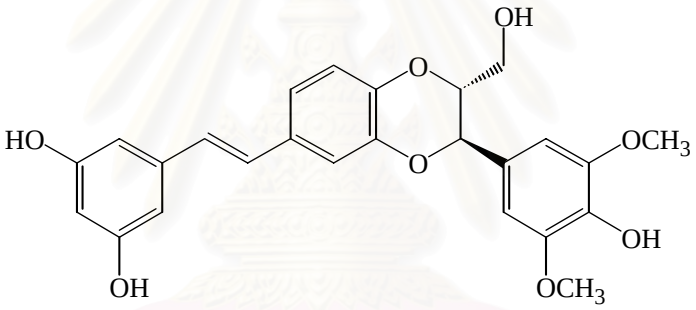
(+)-Catechin [**138**] from the leaves of *Syzygium corynocarpum* and *S. malaccense* (Myrtaceae) showed weak COX-2 inhibitory effect (IC_{50} = 130 μM) (Noreen *et al*., 1998). Cyanidin [**139**] from tart cherries showed COX-2 inhibitory activity at IC_{50} of 60 nM when tested with human recombinant COX-2 (Wang *et al*., 1999a). Apigenin [**140**], kaempferol [**141**] and genistein [**142**] inhibited PGE_2 production by more than 50% at 15 μM. Among them, apigenin [**140**] was the most potent inhibitor of PGE_2 production (IC_{50} = 8.04 μM) (Liang *et al*., 1999).

3.8 Sulfur containing compounds

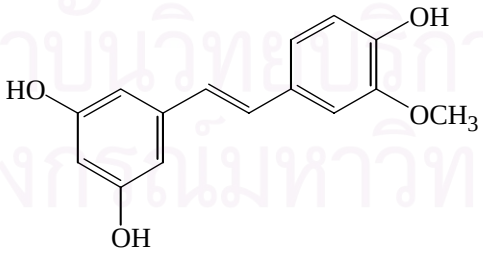
(*Z*)-Ajoene [**143**] and (*E*)-ajoene [**144**] from the methanol extract of *Allium sativum* (Alliaceae) inhibited the release of PGE_2 from LPS-activated RAW 264.7 cells (IC_{50} = 2.4 μM) and inhibited the conversion of exogenous arachidonic acid to PGE_2 at IC_{50} of 3.4 μM (Dirsch and Vollmar, 2001).



[**122**] Platycodin D

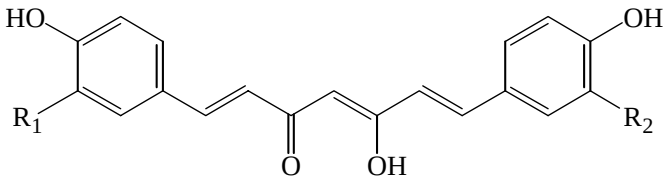


[**123**] Aiphanol

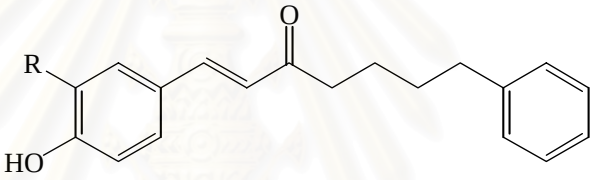


[**124**] Isorhapontigenin

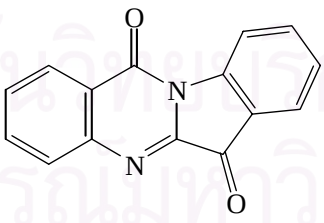
Figure 3 Structures of COX-2 inhibitors from natural sources



R_1	R_2	
OCH_3	OCH_3	[**125**] Curcumin I
H	OCH_3	[**126**] Curcumin II
H	H	[**127**] Curcumin III

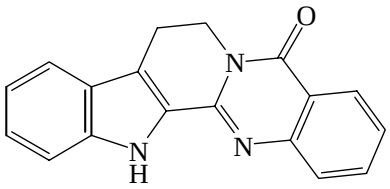


R	
OCH_3	[**128**] Yakuchinone B
OH	[**129**] Demethyl-yakuchinone B

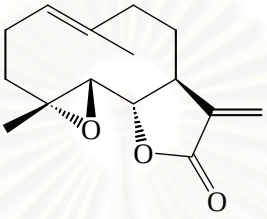


[**130**] Tryptanthrin

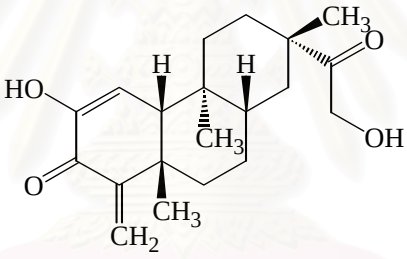
Figure 3 Structures of COX-2 inhibitors from natural sources (continued)



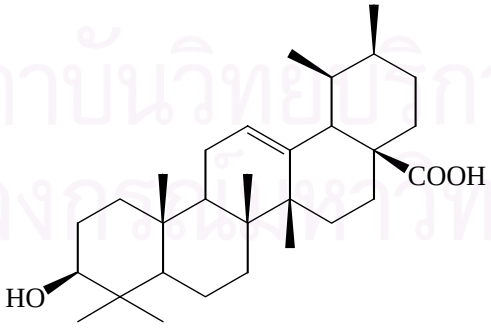
[**131**] Rutaecarpine



[**132**] Parthenolide

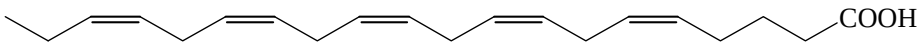


[**133**] Akendo 3

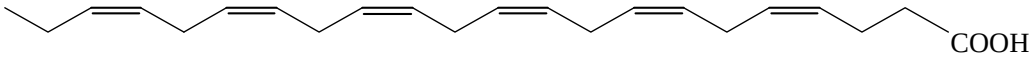


[**134**] Ursolic acid

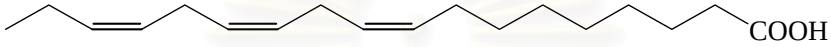
Figure 3 Structures of COX-2 inhibitors from natural sources (continued)



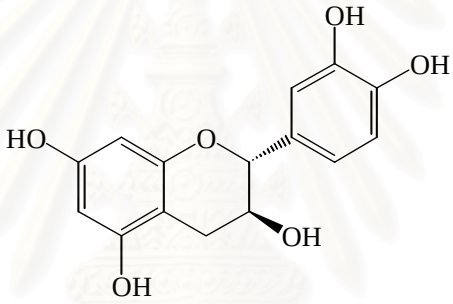
[**135**] EPA



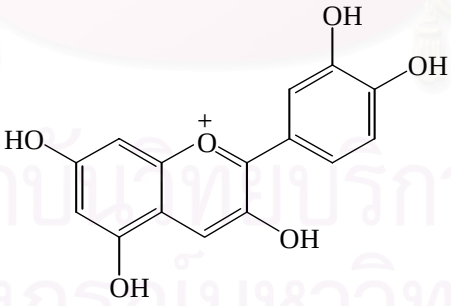
[**136**] DHA



[**137**] α-LNA

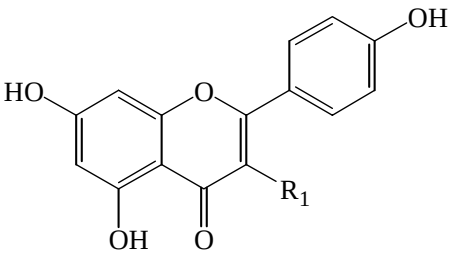


[**138**] (+)-Catechin

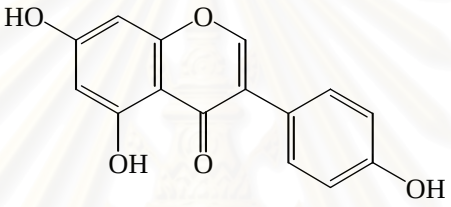


[**139**] Cyanidin

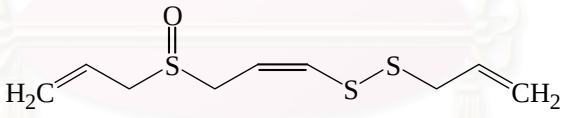
Figure 3 Structures of COX-2 inhibitors from natural sources (continued)



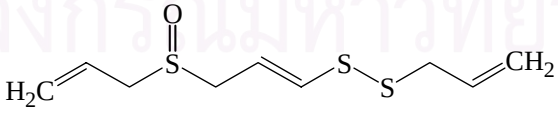
R	
H	[**140**] Apigenin
OH	[**141**] Kaempferol



[**142**] Genistein



[**143**] (*Z*)-Ajoene



[**144**] (*E*)-Ajoene

Figure 3 Structures of COX-2 inhibitors from natural sources (continued)

CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The stem of *Dracaena loureiri* Gagnep. was purchased from Wethchapong Osot drugstore in 1999. Authentication was done by comparison with specimens at the museum of Natural Medicine, Chulalongkorn University. A voucher specimen (KL 062542) is on deposit at the Faculty of Phamaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel $60F_{254}$ (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6 cm
Temperature	:	room temperature (30-35°C)
Detection	:	1. Ultraviolet light at 254 and 365 nm
		2. Anisaldehyde and heating at 105°C for 10 min.

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)

Silica gel 60 (No. 5111) particle size 0.015-0.040 mm (E. Merck)

Packing method : Dry packing

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

Detection : Fractions were examined by TLC observing under UV light at 254 and 365 nm

2.2.2 Flash Column Chromatography

Adsorbent : Silica gel 60 (No. 9385) particle size 0.400-0.063 mm (230-400 mesh ASTM) (E. Merck)

Silica gel 60 (No.5111) particle size 0.015-0.040 mm (E. Merck)

Packing method : Wet packing

Sample loading : The sample was dissolved in a small amount of the eluent and then applied gently on top of the column.

Detection : Fractions were examined by TLC observing under UV light at 254 and 365 nm

2.2.3 Gel Filtration Chromatography

Gel filter	:	Sephadex LH 20 (Pharmacia)
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small volume of the eluent and applied on top of the column.
Detection	:	Fractions were examined by TLC observing under UV light at 254 and 365 nm

2.3 High Pressure Liquid Chromatography (HPLC)

Column	:	Shim-pack PREP-SIL No. 2025810
Flow rate	:	3 ml/min
Mobile phase	:	chloroform-methanol (24: 1)
Sample preparation	:	The sample was dissolved in a small amount of chloroform and filtered through millipore filter paper before injection.
Injection volume	:	1 ml
Pump	:	LC-8A (Shimadzu)
Detector	:	SPD-10A UV Detector (Shimadzu)
Recorder	:	C-R6A Chromatopac (Shimadzu)
Temperature	:	room temperature (30-35°C)

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectra

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

2.4.2 Infrared (IR) Absorption Spectra

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

2.4.3 Mass Spectra

Electron impact mass spectra (EIMS) were measured with a FISONS VG TRIO 2000 mass spectrometer (Department of Chemistry, Faculty of Science, Chulalongkorn University). The High Resolution Fast-Atom Bombardment mass spectrum (HREIMS) was measured with a Finnigan MAT 95 mass spectrometer (Institute of Chemistry, Hohenheim University, Germany).

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

^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H-^{1}H COSY, NOESY, HETCOR, COLOC and HMBC spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

Solvents for NMR spectra were deuterated acetone (acetone-d_6) and deuterated chloroform ($CDCl_3$). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.5 Physical Properties

2.5.1 Melting Points

Melting points were obtained on a Gallenkamp melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Optical Rotation

Optical rotations were measured on a Perkin Elmer 341 polarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.6 Solvents

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

3. Extraction and Isolation

3.1 Extraction

The dried and powdered stem of *Dracaena loureiri* (6 kg) was extracted with hexane (3×20 L) and then filtered. The filtrate was evaporated under reduced pressure to yield a hexane extract (50 g, 0.83 % based on dried weight of stem). The marc was then extracted with ethyl acetate (3×20 L). The obtained extract was evaporated under reduced pressure to give an ethyl acetate extract (450 g, 7.50 % based on dried weight of stem).

Finally, the marc was extracted with methanol (3×20 L). Removal of the organic solvent gave a methanol extract (950 g, 15.83 % based on dried weight of stem). Each extract was subjected to COX-2 inhibitory activity evaluation as described in Section 5.

3.2 Isolation

The ethyl acetate extract, which was found to be active against the COX-2 enzyme (see Section 5), was divided into two equal portions: A and B. Each portion was fractionated by quick column chromatography, using a sintered glass filter column of silica gel 60 (No. 7734) (400 g). Elution was performed in a polarity gradient manner with hexane, ethyl acetate and methanol as the solvents (Table 2).

Table 2 Quick column chromatography of ethyl acetate extract of *D. loureiri*

Portion	Fraction	Ratio (%) of ethyl acetate : hexane	Volume of solvent (ml)
A	1-2	20 : 80	1000
	3-7	30 : 70	2500
	8-12	40 : 60	2500
	13-36	50 : 50	12000
	37-44	100 : 0	4000
	45-48	methanol	2000
B	1-2	20 : 80	1000
	3-13	30 : 70	5500
	14-17	40 : 60	2000
	18-29	50 : 50	6000
	30-37	100 : 0	4000
	38-41	methanol	2000

The eluates obtained from each column were examined by TLC (silica gel, hexane-ethyl acetate, 1: 1). Fractions with similar chromatographic pattern were combined (Tables 3 and 4).

Table 3 Combination of fractions from portion A

Fraction	Combined fractions	Total weight (g)
A	1-2	0.5
B	3-4	1.0
C	5-13	4.4
D	14-16	2.7
E	17-33	52.5
F	34-36	5.9
G	37-38	1.9
H	39-40	9.5
I	41-48	11.0

Table 4 Combination of fractions from portion B

Fraction	Combined fractions	Total weight (g)
J	1-3	1.3
K	4	1.0
L	5	0.9
M	6-12	7.9
N	13-19	17.3
O	20-21	6.6
P	22-29	11.3
Q	30-41	27.4

Fractions D and N, showing similar chromatographic pattern, were combined to give fraction DN.

A portion of DN (8.6 g) was separated by quick column chromatography, using a sintered glass filter column of silica gel (No. 5111, 100 g). Elution was performed in a polarity gradient manner with chloroform and methanol as the solvents (Table 5).

Table 5 Quick column chromatography of DN

Fraction	Ratio (%) of methanol : chloroform	Volume of solvent (ml)
1-3	0 : 100	300
4-28	3 : 97	2500
29-45	6 : 94	1700
46-50	8 : 92	500
51-60	10 : 90	1000
61-62	20 : 80	200
63-64	40 : 60	200
65-66	80 : 20	200
67-70	100 : 0	400

The eluates were examined by TLC (silica gel, $CHCl_3$-MeOH, 23: 2). Fractions with similar chromatographic pattern were combined (Table 6).

Table 6 Combination of fractions from DN

Fraction	Combined fractions	Weight (g)
DN1	1-3	0.059
DN2	4-5	0.005
DN3	6-8	0.018
DN4	9-10	0.506

Table 6 Combination of fractions from DN (continued)

Fraction	Combined fractions	Weight (g)
DN5	11	1.579
DN6	12	1.778
DN7	13	0.673
DN8	14-15	0.555
DN9	16	0.336
DN10	17-31	1.487
DN11	32-41	0.919
DN12	42	0.019
DN13	43-49	0.142
DN14	50-51	0.085
DN15	52-54	0.140
DN16	55-60	0.067
DN17	61-70	0.166

3.2.1 Isolation of Compounds DL-A, DL-B and DL-C

Compound DL-A was obtained as colorless needles from fraction DN-5 through recrystallization from a mixture of hexane-$CHCl_3$ (381 mg, 1.48×10^{-2} % based on dried weight of stem; R_f 0.36, silica gel, $CHCl_3$-MeOH, 9: 1). It was identified as 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone [**101**].

The mother liquor was dried and fractionated on a column using silica gel 60 (No. 5111) (240 g) as the adsorbent. Elution was performed in a polarity gradient manner with chloroform and methanol. Fractions of 50 ml each were collected. Fractions with similar chromatographic pattern were combined to give

twelve fractions: DN51 (1 mg), DN52 (1 mg), DN53 (41 mg), DN54 (4 mg), DN56 (4 mg), DN57 (58 mg), DN58 (219 mg), DN59 (58 mg), DN510 (36 mg), DN511 (21 mg) and DN512 (77.2 mg).

Fraction DN53 (41 mg) was separated by HPLC using a normal phase column (Shim pack PREP-SIL) with $CHCl_3$-MeOH (24: 1) and UV-VIS detection to give compound DL-B as colorless needles (11 mg, 8.81×10^{-4} % based on dried weight of stem; R_f 0.4, silica gel, $CHCl_3$-MeOH, 24: 1). It was later identified as pterostilbene [**51**].

Fraction DN58 (219 mg) was fractionated on a column using silica gel 60 (No. 9385) (100 g) as the adsorbent. Isocratic elution with $CHCl_3$ - MeOH(99: 1) was performed. Twenty-four fractions of 30 ml each were collected. Fractions showing similar chromatographic pattern were combined. Fractions DN58-5 (118 mg) were combined and further separated by gel filtration chromatography, using a column of Sephadex LH-20 with $CHCl_3$-MeOH (1: 1) as the eluent. Twelve 10 ml- fractions were collected. Fraction DN58-5-3 (69 mg) was reseparated by gel filtration chromatography, using a column of Sephadex LH-20 with $CHCl_3$-MeOH (1: 1) to give 41 mg (3.28×10^{-3} % based on dried weight of stem) of compound DL-C as white needles (R_f 0.34, silica gel, hexane-ethyl acetate, 1: 1). It was later identified as loureirin B [**53**].

3.2.2 Isolation of compounds DL-D, DL-E and DL-F

Fraction DN10 (1.5 g) was separated by gel filtration chromatography using a column of Sephadex LH-20 (100 g, 2.5×80 cm) with methanol as the eluent. Thirty-three fractions (30 ml each) were collected and examined by TLC (silica gel, $CHCl_3$-MeOH, 9: 1). Fractions with similar chromatographic pattern were combined to yield twelve major fractions: DN10-1 (31 mg), DN10-2 (14 g), DN10-3

(146 mg), DN10-4 (176 mg), DN10-5 (757 mg), DN10-6 (123 mg), DN10-7 (171 mg), DN10-8 (17 mg), DN10-9 (7 mg), DN10-10 (10 mg), DN10-11 (7 mg) and DN10-12 (16 mg).

Compound DL-D (171 mg, 6.63×10^{-3} % based on dried weight of stem) was obtained as orange needles from fraction DN10-7 (R_f 0.23, silica gel, $CHCl_3$-MeOH, 9: 1). It was later identified as pinostilbene [**145**].

Compound DL-E (757 mg, 2.93×10^{-2} % based on dried weight of stem) was obtained as pink needles from fraction DN10-5 (R_f 0.23, silica gel, $CHCl_3$-MeOH, 9: 1). It was later identified as 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**83**].

Fraction DN10-3 (146 mg) was separated by gel filtration, using a column of Sephadex LH-20 (100 g, 2.5×80 cm) with methanol as the eluent. Twenty fractions (30 ml each) were collected and examined by TLC (silica gel, $CHCl_3$-MeOH, 9: 1). Fractions showing similar chromatographic pattern were combined to yield six major fractions: DN10-3-1 (1 mg), DN10-3-2 (3 mg), DN10-3-3 (12 mg), DN10-3-4 (112 mg), DN10-3-5 (15 mg) and DN10-3-6 (1 mg). Evaporation of fraction DN10-3-4 under reduced pressure gave 112 mg (4.43×10^{-3} % based on dried weight of stem) of compound DL-F as an orange amorphous powder (R_f 0.26, silica gel, $CHCl_3$-MeOH, 9: 1). This compound was identified as 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone [**100**].

3.2.3 Isolation of compounds DL-G and DL-H

Fraction DN11 (454 mg) was fractionated on a column using silica gel 60 (No. 5111) (220 g) as the adsorbent. Elution was performed in an isocratic manner with $CHCl_3$-MeOH- (93: 7). The eluates were examined by TLC (silica gel, $CHCl_3$-MeOH, 9: 1). Fractions (50 ml each) showing similar

chromatographic pattern were combined to yield thirteen major fractions: DN11-1 (2 mg), DN11-2 (8 mg), DN11-3 (52 mg), DN11-4 (14 mg), DN11-5 (120 mg), DN11-6 (43 mg), DN11-7 (103 mg), DN11-8 (24 mg), DN11-9 (46 mg), DN11-11 (7 mg), DN11-12 (1 mg) and DN11-13 (31 mg).

Fraction DN11-5 (120 mg) was separated by gel filtration, using a column of Sephadex LH-20 (100 g, 2.5×80 cm) with methanol as eluent. Nineteen fractions (20 ml each) were collected and were examined by TLC (silica gel, $CHCl_3$-MeOH, 9: 1). Fractions DN11-5-2 (52 mg) were combined and further separated by gel filtration chromatography, using a column of Sephadex LH-20 with $CHCl_3$-MeOH(1: 1) as the eluent. Seventeen fractions (10 ml each) were collected and examined by TLC (silica gel, $CHCl_3$-MeOH, 9: 1). Fractions DN11-5-2-3 (R_f 0.2, silica gel, $CHCl_3$-MeOH, 9: 1) were combined and dried to give 30 mg (2.35×10^{-3} % based on dried weight of stem) of compound DL-G as a yellowish amorphous powder. This compound has a new structure, being identified as 3,5,7-trihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**146**].

Fraction DN11-7 (103 mg) was separated by gel filtration chromatography, using a column of Sephadex LH-20 (100 g, 2.5×80 cm) with methanol as the eluent. Twelve fractions (20 ml each) were collected, examined by TLC (silica gel, $CHCl_3$-MeOH, 9: 1) and combined to give five major fractions: DN11-7-1 (1 mg), DN11-7-2 (2 mg), DN11-7-3 (79 mg), DN11-7-4 (18 mg) and DN11-7-5 (4 mg).

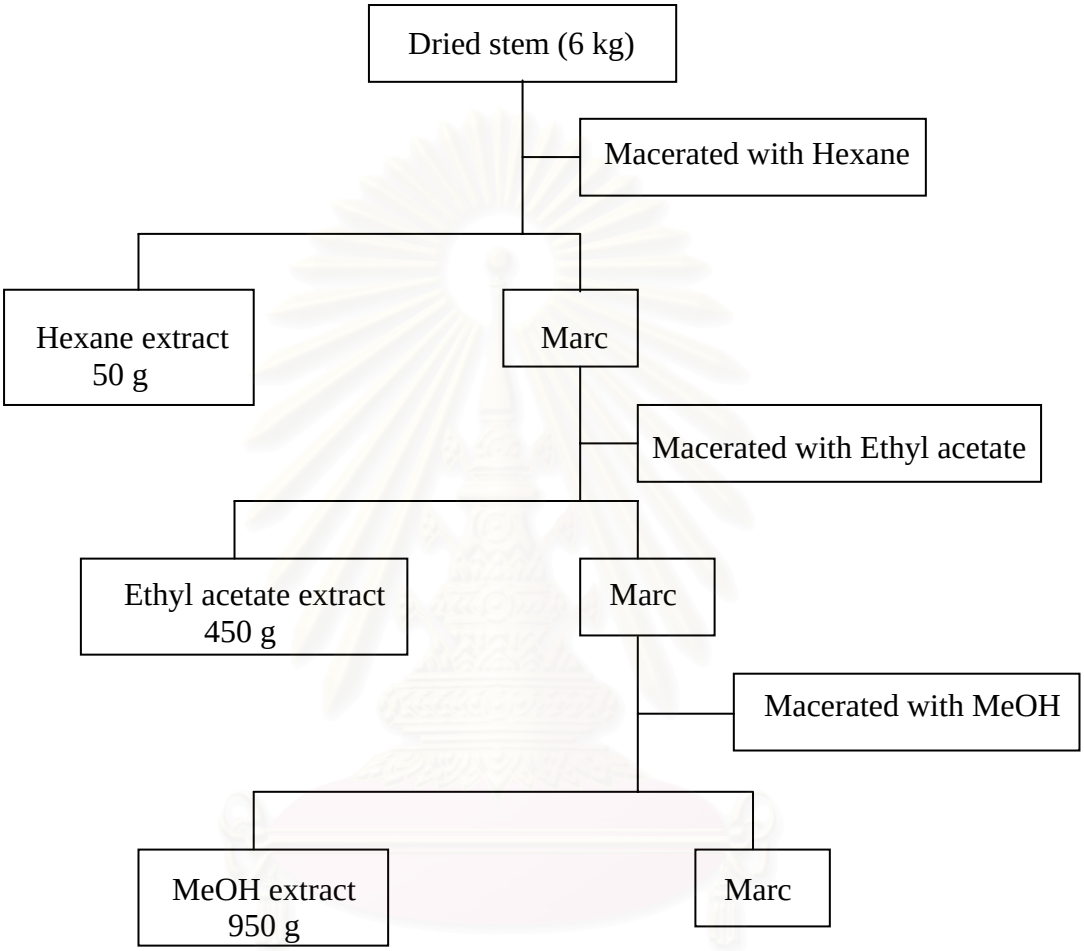
Compound DL-H (79 mg, 6.20×10^{-3} % based on dried weight of stem) was obtained as red needles from fraction DN11-7-3 (R_f 0.18, silica gel, $CHCl_3$-MeOH, 9: 1). It was identified as loureirin D [**94**].

3.2.4 Isolation of compound DL-I

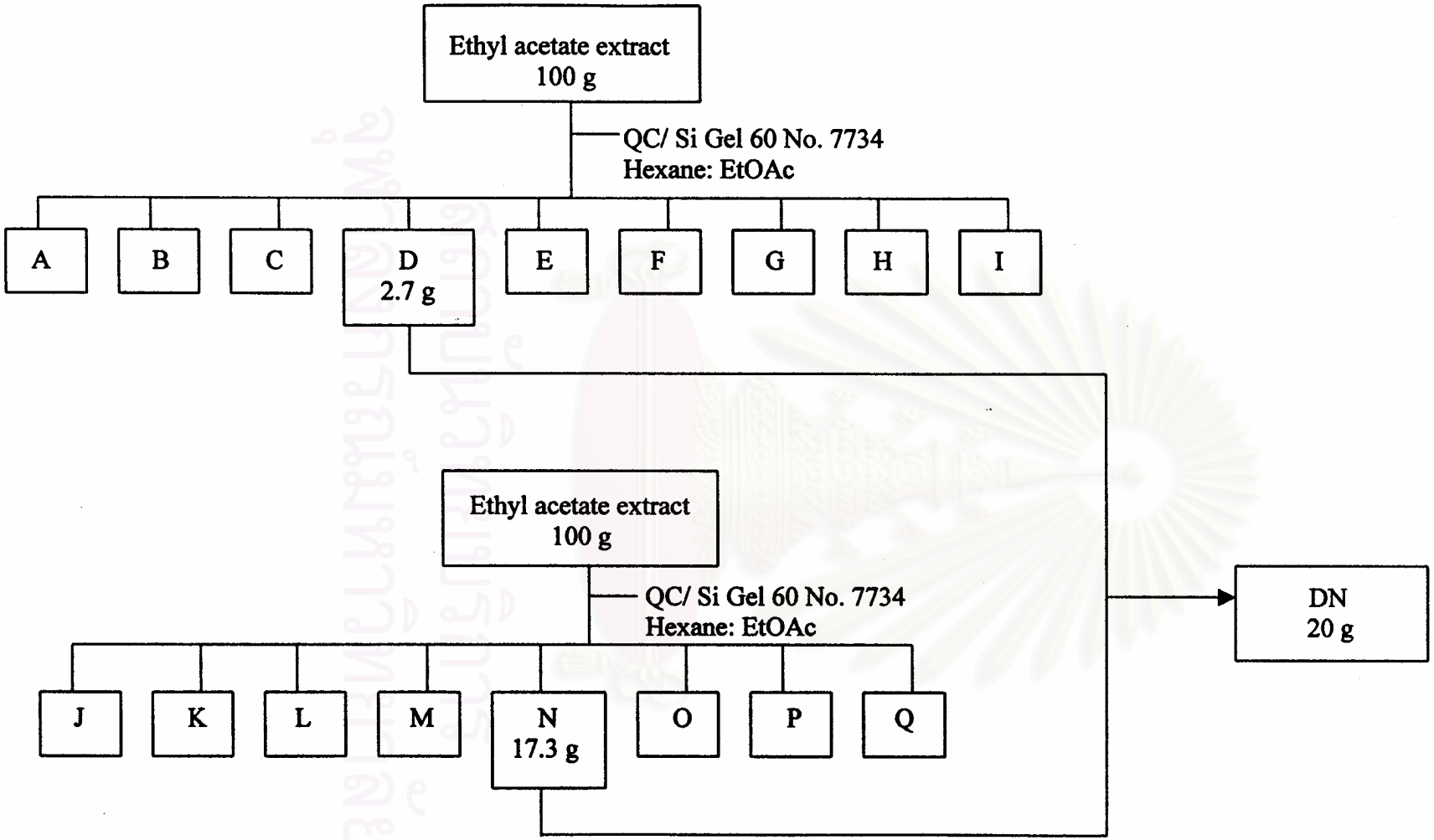
Fraction DN15 (123 mg) was separated by gel filtration chromatography, using a column of Sephadex LH-20 (100 mg, 2.5×80 cm) with acetone as the eluent. Twenty-seven fractions (30 ml each) were collected, examined by TLC (silica gel, $CHCl_3$-MeOH, 17: 3) and combined to give seven major fractions: DN15-1 (14 mg), DN15-2 (8 mg), DN15-3 (14 mg), DN15-4 (40 mg), DN15-5 (36 mg), DN15-6 (11 mg) and DN15-7 (1 mg).

Fraction DN15-4 (40 mg) was separated by gel filtration chromatography, using a column of Sephadex LH-20 (100 g, 2.5×80 cm) with acetone as the eluent. Thirty fractions (5 ml each) were collected, examined by TLC (silica gel, $CHCl_3$-MeOH, 17: 3) and combined to yield six major fractions: DN15-4-1 (1 mg), DN15-4-2 (5 mg), DN15-4-3 (9 mg), DN15-4-4 (12 mg), DN15-4-5 (2 mg) and DN15-4-6 (4 mg). Evaporation of fraction DN15-4-4 under reduced pressure gave 12 mg of compound DL-I as orange needles (R_f 0.32, silica gel, $CHCl_3$-MeOH, 17: 3).

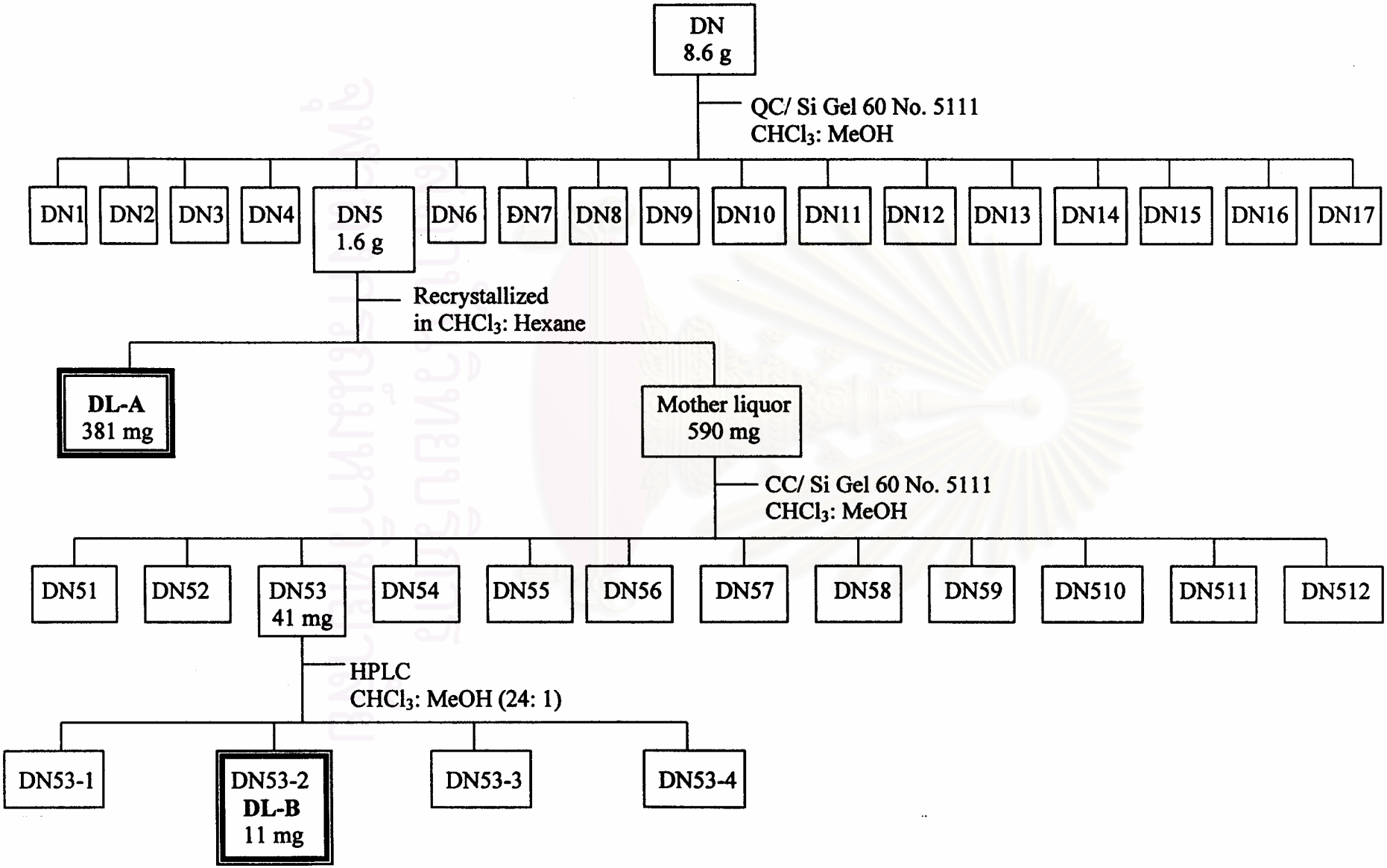
Fraction DN15-5 (36 mg) was separated by gel filtration chromatography in a similar manner to give DL-I (30 mg). It was later identified as resveratrol [**43**]. The total weight of fraction DN15-4-4 and DN15-5 to give DL-I (42 mg, 1.85×10-3 % based on dried weight of stem).



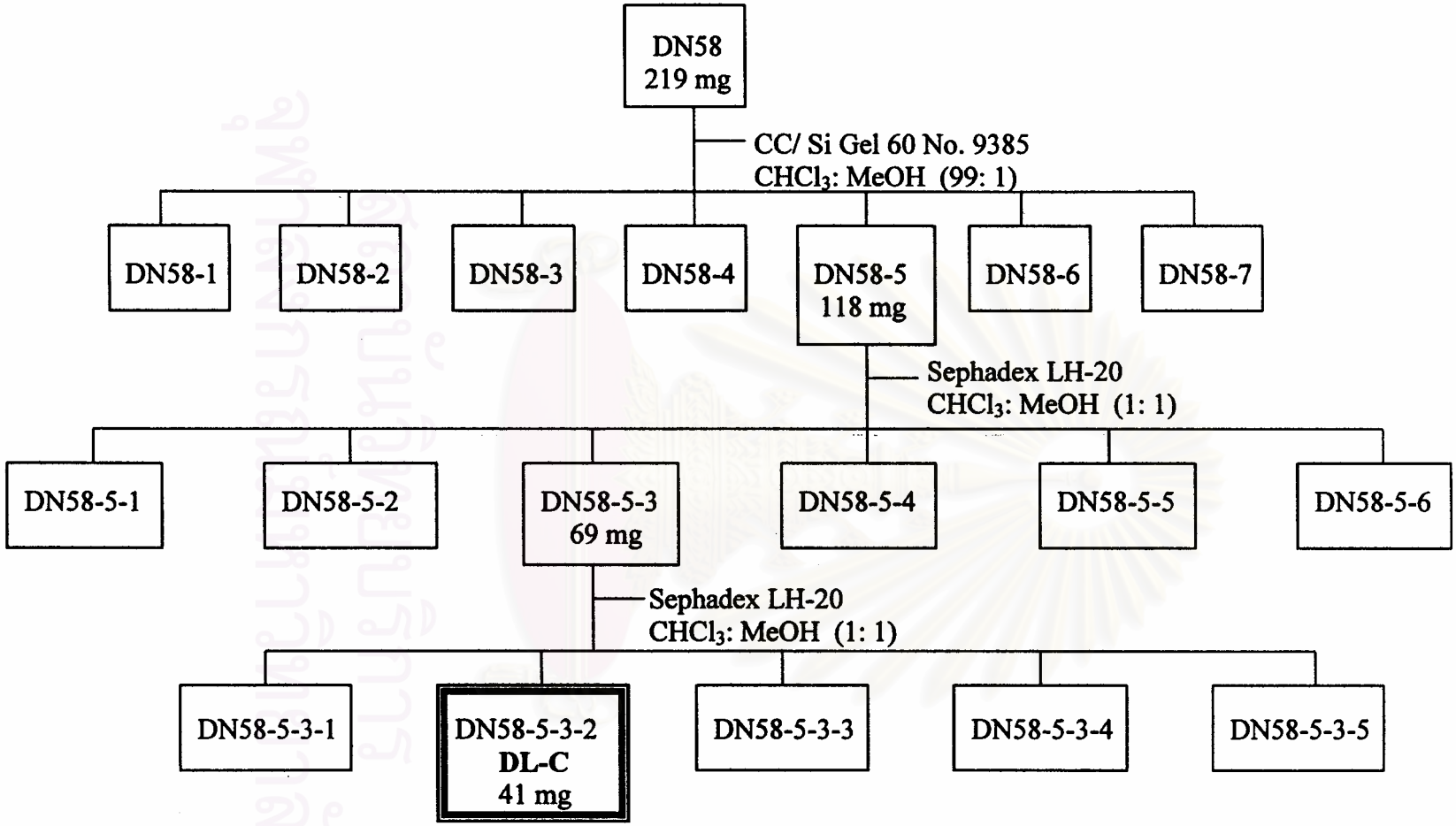
Scheme 1 Extraction scheme of *Dracaena loureiri* stem



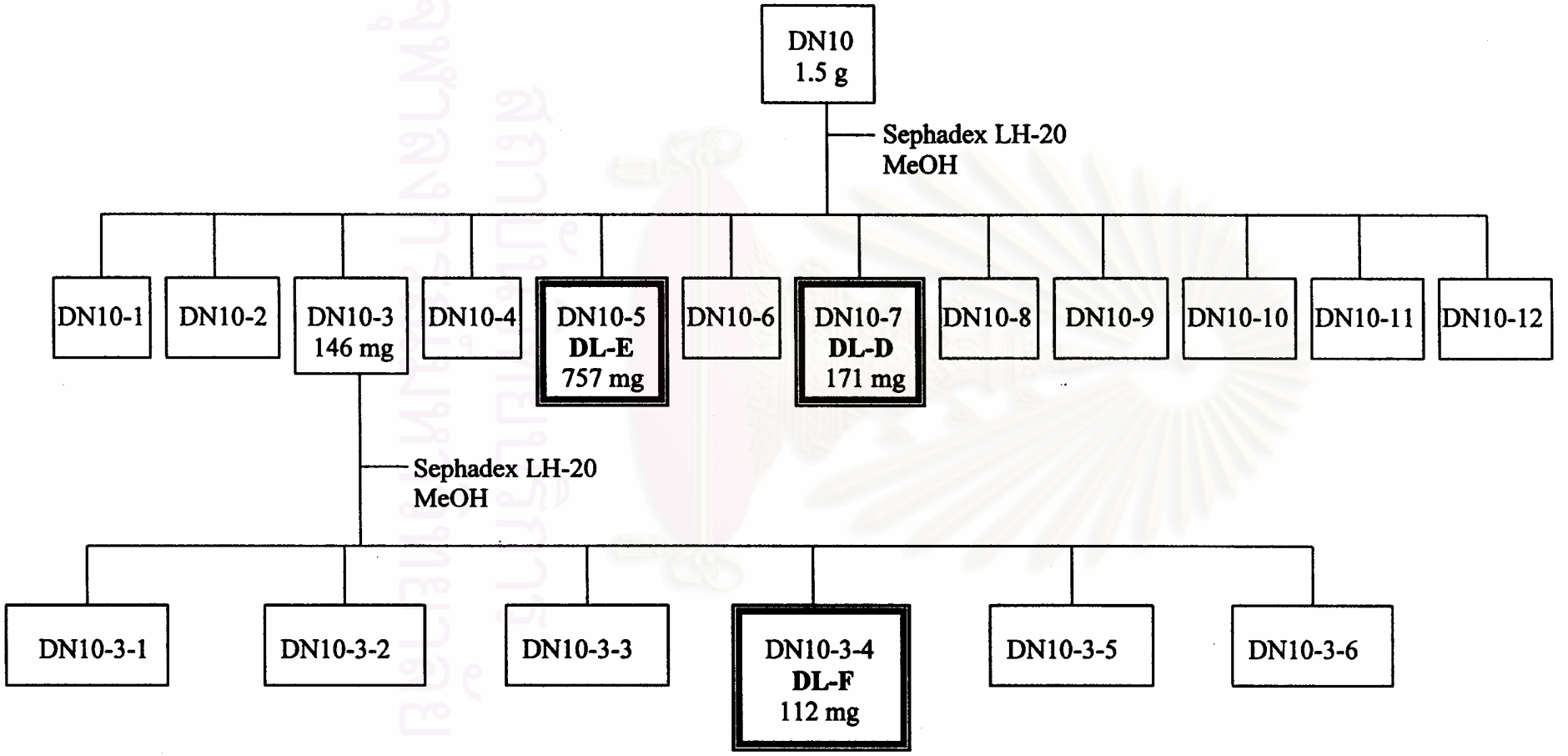
Scheme 2 Isolation scheme of ethyl acetate extract



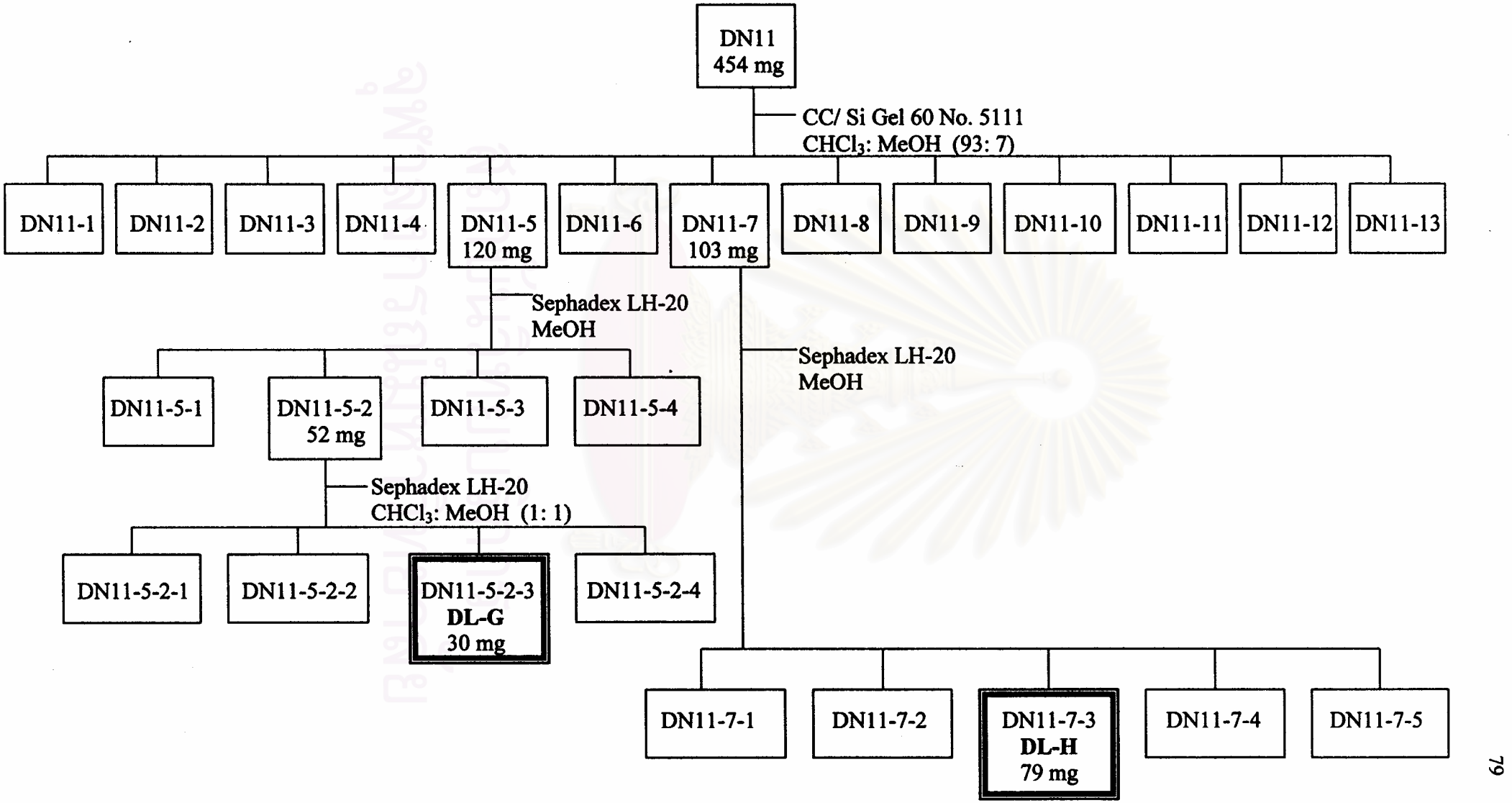
Scheme 3 Isolation scheme of DN of ethyl acetate extract



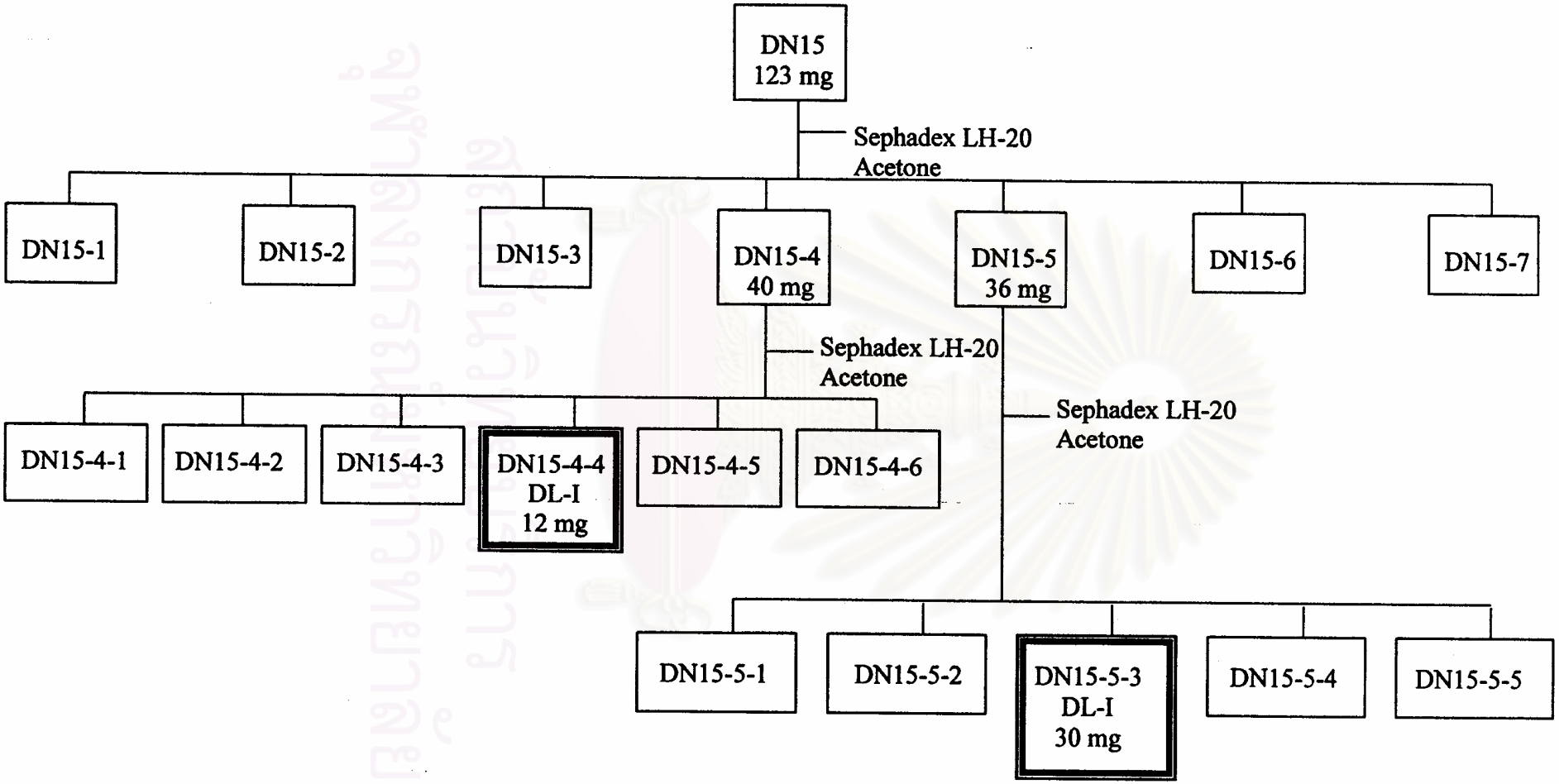
Scheme 4 Isolation scheme of DN58 of DN



Scheme 5 Isolation scheme of DN10 of DN



Scheme 6 Isolation scheme of DN11 of DN



Scheme 7 Isolation scheme of DN15 of DN

4. Physical and Spectral data of Isolated Compounds

4.1 Compound DL-A

Compound DL-A was obtained as colorless needles (381 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 7

$_{302}$ (M^+, 10), 181 (8), 167 (100), 154 (68), 121 (30)

UV : λ_{max} nm (log ε), in methanol; Figure 5

207 (4.43), 223 (sh, 4.10), 278 (4.03)

IR : ν_{max} cm^{-1}, KBr disc; Figure 6

3410 (br), 1651, 1621, 1603, 1512, 1251, 1170, 1151, 1109, 1053, 841, 818

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 9; Figure 8

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 9; Figure 9

4.2 Compound DL-B

Compound DL-B was obtained as colorless needles (11 mg). It was soluble in chloroform.

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

256 (M^+, 4), 152 (15), 115 (32), 98 (19), 69 (25), 57 (39), 43 (100)

UV : λ_{max} nm (log ε), in methanol; Figure 10

$_{218}$ (4.27), 238 (sh, 4.10), 306 (4.39), 319 (4.37)

IR : ν_{max} cm^{-1}, KBr disc; Figure 11

3415 (br), 2940, 2839, 1596, 1514, 1204, 1155, 1067, 961, 835

^{1}H-NMR : δ ppm, 300 MHz, in chloroform-d_6, Table 10; Figure 13

^{13}C-NMR : δ ppm, 75 MHz, in chloroform-d_6, Table 10; Figure 15

4.3 Compound DL-C

Compound DL-C was obtained as white needles (41 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 20

316 (M^+, 0.1), 195 (9), 121 (100), 107 (9), 93 (23), 77 (10), 65 (24)

UV : λ_{max} nm (log ε), in methanol; Figure 18

207 (4.47), 223 (sh, 4.13), 277 (4.04)

IR : ν_{max} cm^{-1}, KBr disc; Figure 19

3423 (br), 3121, 2942, 2836, 2630, 1649, 1605, 1575, 1501, 1292, 1229, 1207, 1180, 1153, 1126, 1050, 827

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 11; Figure 21

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 11; Figure 22

4.4 Compound DL-D

Compound DL-D was obtained as orange needles (171 mg). It was soluble in acetone.

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

242 (M^+, 100), 241 (23), 181 (15), 134 (23), 112 (19), 98 (68), 84 (46), 74 (42), 57 (46), 43 (46)

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

217 (4.25), 238 (sh, 4.07), 305 (4.37), 318 (4.35)

IR : ν_{max} cm^{-1}, KBr disc; Figure 24

3438 (br), 3023, 2946, 2842, 1606, 1594, 1195, 1153, 1514, 1056, 961, 834, 803, 681

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 12; Figure 26

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 12; Figure 28

4.5 Compound DL-E

Compound DL-E was obtained as pink needles (757 mg). It was soluble in acetone.

$[\alpha]^{20}_{D}$: -25.7° (MeOH; 0.4 g/ 100 ml)

EIMS : *m/z* (% relative intensity); Figure 33

286 (M^+, 0.2), 179 (5), 152 (7), 133 (10), 124 (12), 107 (100), 77 (31), 69 (15)

UV : λ_{max} nm (log ε), in methanol; Figure 31

212 (4.35), 225 (sh, 4.29), 289 (4.23), 328 (3.59)

IR : ν_{max} cm^{-1}, KBr disc; Figure 32

3429 (br), 1643, 1515, 1263, 1161, 1108, 1074, 1029, 828

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 13; Figure 34a and 34b

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 13; Figure 35

4.6 Compound DL-F

Compound DL-F was obtained as an orange amorphous powder (112 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 38

302 (M^+, 21), 181 (8), 167 (100), 154 (22), 137 (17), 121 (55), 107 (18), 93 (10)

UV : λ_{max} nm (log ε), in methanol; Figure 36

209 (4.44), 223 (sh, 4.26), 278 (4.20)

IR : ν_{max} cm^{-1}, KBr disc; Figure 37

3415 (br), 1661, 1604, 1511, 1163, 1119, 842, 819

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 14; Figure 39

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 14; Figure 40

4.7 Compound DL-G

Compound DL-G was obtained as a yellowish amorphous powder (30 mg). It was soluble in acetone.

$[\alpha]^{20}_{D}$: -128° (MeOH; 0.1 g/ 100 ml)

Melting Point : 109-110°C

EIMS : *m/z* (% relative intensity); Figure 43

302 (M^+, 41), 284 (5), 196 (100), 153 (87), 107 (98), 77 (32), 69 (24), 43 (14)

UV : λ_{max} nm (log ε), in methanol; Figure 41

214 (4.46), 224 (sh, 4.41), 292 (4.32), 330 (3.73)

IR : ν_{max} cm^{-1}, KBr disc; Figure 42

3416 (br), 1643, 1593, 1515, 1302, 1275, 1256, 1163, 1119, 1074, 1030, 835

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 15; Figure 44

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 15; Figure 47

4.8 Compound DL-H

Compound DL-H was obtained as red needles (79 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 52

288 (M^+, 25), 269 (2), 153 (89), 140 (67), 121 (100), 107 (17), 93 (26), 77 (16), 69 (45), 65 (45), 43 (38)

UV : λ_{max} nm (log ε), in methanol; Figure 50

207 (4.42), 223 (sh, 4.08), 279 (4.01)

IR : ν_{max} cm^{-1}, KBr disc; Figure 51

3414 (br), 2943, 2843, 1654, 1628, 1600, 1514, 1473, 1289, 1252, 1212, 1169, 1151, 1100, 836

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 16; Figure 53

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 16; Figure 54

4.9 Compound DL-I

Compound DL-I was obtained as orange needles (42 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 57

228 (M^+, 100), 227 (25), 211 (7), 181 (16), 153 (7), 152 (10), 115 (16), 91 (10), 77 (9), 69 (18), 55 (20)

UV : λ_{max} nm (log ε), in methanol; Figure 55

217 (4.22), 237 (sh, 4.02), 304 (4.35), 318 (4.32)

IR : ν_{max} cm^{-1}, KBr disc; Figure 56

3306 (br), 3023, 1607, 1591, 1514, 1385, 1155, 966, 835

^{1}H-NMR : δ ppm, 300 MHz, in acetone-d_6, Table 17; Figure 58

^{13}C-NMR : δ ppm, 75 MHz, in acetone-d_6, Table 17; Figure 59

5. Evaluation of COX Inhibitory Activity

5.1 Materials and Methods

5.1.1 Cell Culture and Treatment

Stock DMEM

All tissue culture medium and supplements were purchased from Gibco BRL. Ten grams of Dulbecco's modified Eagle medium (DMEM) powder and 49.3 ml of 7.5 % $NaHCO_3$ were added to distilled water. Medium was adjusted to 1 liter with distilled water after pH of 7-7.4 was reached by adding 1 N NaOH. Medium was then filter sterilized with 0.2 μM nylon membrane. Ten ml of filter sterilized 200 mM L-glutamine was then added to this stock solution.

Cell Culture Medium

For growing and maintaining cells, stock DMEM was supplemented with fetal bovine serum (10 % final concentration), 50 mg/ml ascorbic acid, 10 ml/l non-essential amino acid and 800 μg/l Hygromycin B. For drug treatment experiments, cells were grown in the same media without Hygromycin B.

Treatment of Cells

Immortalized murine COX-$1^{-/-}$ and COX-$2^{-/-}$ cells (Kirtikara, Swangkul and Ballou, 2001) were seeded at 1×10^5 cells/ml in DMEM in 96-well flat-bottomed tissue culture plates, 83 μl/well, and incubated in a humidified incubator with 5 % CO_2 for 72 h. Subsequently, cells were washed gently with DMEM without fetal bovine serum and incubated with serum-free DMEM containing vehicle, plant extracts or pure compounds for 30 minutes. Medium was replaced with fresh serum-free DMEM containing vehicle, plant extracts or pure compounds and 2 μM A23187, a calcium ionophore. After additional 30 minutes of incubation, medium from each well was collected and placed in -80°C freezer until further use.

5.1.2 Preparation of Test Samples in Dimethyl Sulfoxide (DMSO)

Pure dimethyl sulfoxide (DMSO) was used to initially solubilize the test compounds at 1×10^{-1} g/ml. Sample (1×10^{-2} g/ml in 10 % DMSO) was subsequently prepared and later diluted before adding to culture medium to yield various concentrations of extracts. Final DMSO concentration in the medium was 0.1 %.

5.1.3 Radioimmuno assay of Prostaglandin E_2

Materials

Anti-PGE_2 antibody was purchased from Sigma Chemicals and ^{3}H-PGE_2 was from Amersham.

Preparation of Solutions

RIA buffer was prepared by adding 22.8 g K_2HPO_4, 13.6 g KH_2PO_4, 9 g NaCl, and 1 g sodium azide in total volume of 1 liter of distilled water. Subsequently, 1 g of gelatin was dissolved in 1 liter of this solution at 37°C until completely solubilized. The solution was kept at 4°C.

Charcoal dextran solution was prepared by adding 2 g dextrans (T-70) in 1 liter of RIA buffer at 37°C. Twenty grams of charcoal was later added and mixed thoroughly.

Stock anti-PGE_2 was prepared by adding 5 ml of 0.01 M sodium phosphate buffer saline, pH 7.4, containing 0.1 % BSA and 0.1 % sodium azide into one vial of lyophilized powder. The vial was rotated gently until the powder was dissolved. In a RIA assay, anti-PGE_2 working solution was prepared by diluting antiserum stock solution 10 fold with the buffer used to prepare the stock solution.

RIA (Radioimmuno assay)

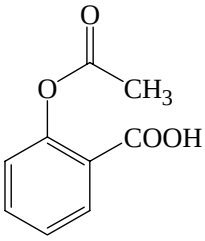
Fifty microliters of media from each well were placed in the 1.5 ml microfuge tubes containing 50 μl ^{3}H-PGE_2. Fifty microliters of anti-PGE_2 working solution was then added to the tube. Two “blank” tubes containing all solutions similar to above sample tubes except replacing anti-PGE_2 with RIA buffer were prepared. Similarly, two “zero” tubes containing DMEM instead of medium from well were prepared. All tubes were kept on ice and subsequently incubated at 4°C overnight. Then, 100 μl of coal charcoal/ dextran were added to tubes that were being kept on ice. After 15 minutes, tubes were centrifuged at 1500 g for 10 minutes at 4°C. The amount of radioactive material in the supernatant were measured using a Packard scintillation counter.

5.1.4 Calculation of PGE_2 Level

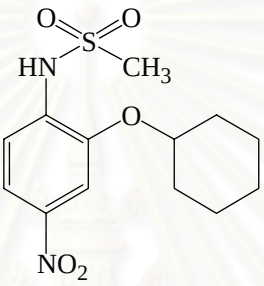
PGE_2 levels were calculated from the following equation for percent binding and compared with the standard curve of known PGE_2.

$$\% \text{ binding} = \frac{(\text{average CPMA-average blank}) \times 100}{(\text{average zero-average blank})}$$

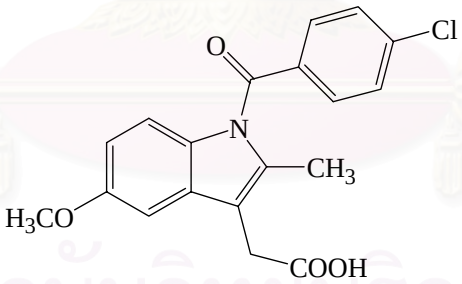
The levels of PGE_2 produced in these COX-1 and COX-2 cell lines correspond to the activity of COX-2 and COX-1 enzymes, respectively. DMSO (0.1%) was used as a control for 100 % COX activity. Aspirin [**147**], NS-398 [**148**] and indomethacin [**149**] were used as positive controls.



[**147**] Aspirin



[**148**] NS-398



[**149**] Indomethacin

Figure 4 Structures of COX inhibitors used as positive controls

5.1.5 Determination of IC_{50}

Samples were diluted to 8 concentrations, including 10^{-9}, 10^{-8}, 3.3×10^{-8}, 10^{-7}, 3.3×10^{-7}, 10^{-6}, 3.3×10^{-6} and 10^{-5} g/ ml. Each of these concentrations was tested for the COX inhibitory activity as described above. The % COX inhibition of each concentration was calculated and used to plot a graph. The IC_{50} value of each pure compound was then obtained from the graph.

5.2 Results

The ethyl acetate extract and the methanol extract were initially tested for the ability to inhibit COX-2. As shown in Table 7, both extracts showed very strong inhibitory effect on COX-2 enzyme. Further separation of the ethyl acetate extract yielded 9 compounds, which were subsequently tested for inhibitory effects on both COX-1 and COX-2. The percentages COX-1 and COX-2 inhibition of pure compounds were shown in Table 8. Compounds DL-B, DL-D and DL-I were the most potent COX-2 and COX-1 inhibitors. The weak COX-2 inhibitors were DL-A, DL-E, DL-F and DL-H, and the inactive compounds were DL-C and DL-G.

Table 7 Percentages of COX-2 inhibition by the extract from *Dracaena loureiri*

Crude extracts	COX-2 inhibition (%)
Hexane	not tested
Ethyl acetate	97
Methanol	97

Table 8 Percentages of COX inhibition by the pure compounds isolated from *Dracaena loureiri*

Compound	%Inhibition at 10 μg/ml[a]		IC_{50} (μM)	
	COX-1	COX-2	COX-1	COX-2
2,4′-Dihydroxy-4,6-dimethoxy-dihydrochalcone (DL-A) [**101**]	33.6	31.8	-	-
Pterostilbene (DL-B) [**51**]	94.3	90.5	4.84±1.16 (3)[c]	1.29±0.74 (3)
Loureirin B (DL-C) [**53**]	NI[b]	3.2	-	-
Pinostilbene (DL-D) [**145**]	97.6	96.7	4.92±3.73 (3)	2.21±1.02(3)
5,7-Dihydroxy-3-(4-hydroxy-benzyl)-4-chromanone (DL-E) [**83**]	53.6	51.1	-	-
4,4′-Dihydroxy-2,6-dimethoxy-diydrocalcone (DL-F) [**100**]	74.4	24.3	-	-
3,5,7-Trihydroxy-3-(4-hydroxy-benzyl)-4-cromanone (DL-G) [**146**]	NI	3.3	-	-
Loureirin D (DL-H) [**94**]	40.4	35.5	-	-
Resveratrol (DL-I) [**43**]	82.2	95.7	2.61±1.19 (3)	2.16±0.93 (3)
Aspirin [**147**]	93.1	35.6	11.41±3.71 (6)	19.80±11.2 (4)
NS-398 [**148**]	-	-	NI	0.01±0.01 (4)
Indomethacin [**149**]	-	-	0.005±0.003 (7)	0.006±0.002 (9)

[a]Compounds with > 80% inhibition were further analyzed for IC_{50} value.

[b]NI = No inhibition.

[c]Mean ± SE (n)

CHAPTER IV

RESULTS AND DISCUSSION

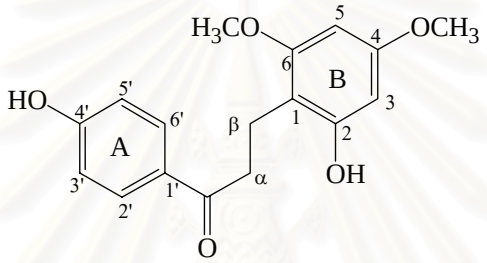
The dried stem of *Dracaena loureiri* Gagnep. (6 kg) was extracted with hexane, ethyl acetate and methanol to give a hexane extract (50 mg), an ethyl acetate extract (450 mg) and a methanol extract (950 mg), respectively. The dried ethyl acetate extract was separated by repetitive chromatography to afford nine compounds. The structures of the isolates were determined based on their UV, IR, NMR and MS data, and subsequently confirmed by comparison of these values with those reported in the literature. The cyclooxygenase-2 (COX-2) inhibitory activity of each pure compound was evaluated.

1. Structure Determination of Isolated Compounds

1.1 Structure Determination of Compound DL-A

Compound DL-A was obtained as colorless needles. The UV spectrum (Figure 5) showed maximal absorptions at 207, 223 (sh) and 278 nm. The IR spectrum (Figure 6) exhibited absorption bands at 3410 (OH), 1651 (C=O), 1603 (>C=C<), 1512 (aromatic ring) and 1170 (C-O stretching of ether) cm^{-1}. The EIMS (Figure 7) revealed a $[M]^+$ ion at *m/z* 302, consistent with the molecular formula $C_{17}H_{18}O_5$. A significant peak was found at *m/z* 167 $[M\text{-}C_8H_7O_2]^+$. DL-A was identified as 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone [**101**], a retrodihydrochalcone previously isolated from *Dracaena loureiri* (Ichikawa *et al*., 1997). The ^1H and ^{13}C NMR data of DL-A are in good agreement with those of **101** (Table 9). The ^1H-NMR spectrum (Figure 8) exhibited two triplet signals at δ 2.90 and δ 3.16

assignable to H-β and H-α (J = 7.1 Hz). Two methoxyl groups appeared at δ 3.71 and δ 3.78. The *meta*-coupled doublet (J = 3.0 Hz) at δ 6.10 was assigned to H-3 and H-5. The *ortho*-coupled doublets (J = 8.7 Hz) at δ 6.92 (2H, H-3′ and H-5′) and δ 7.95 (2H, H-2′ and H-6′) revealed *p*-substitution on ring A. The ^{13}C-NMR and DEPT spectra (Figure 9) showed two methoxyl groups at δ 55.26 and δ 55.76, two methylene carbons for of C-β (δ 18.55) and C-α (δ 38.95), in addition to six methine and seven quaternary carbons.



[**101**]

Table 9 ^{1}H and ^{13}C NMR spectral data of compound DL-A (in acetone-d_6) and 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone (in acetone-d_6)

Position	Compound DL-A		2,4′-dihydroxy-4,6-dimethoxydihydrochalcone	
	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)
1	-	108.93	-	109.17
2	-	156.87	-	157.26
3	6.10 (d, 3.0)	94.84	6.10 (s)	95.06
4	-	159.75	-	160.14
5	6.10 (d, 3.0)	91.03	6.10 (s)	91.23

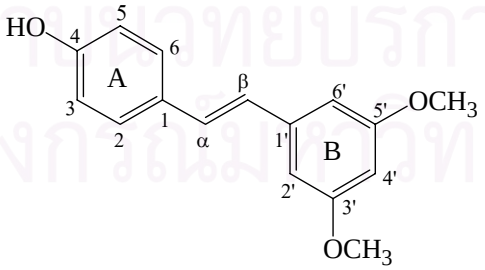
Table 9 ^{1}H and ^{13}C NMR spectral data of compound DL-A (in acetone-d_6) and 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone (in acetone-d_6) (continued)

Position	Compound DL-A		2,4′-dihydroxy-4,6-dimethoxydihydrochalcone	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
6	-	160.21	-	160.60
1′	-	129.58	-	129.90
2′	7.95 (d, 8.7)	131.27	7.95 (d, 9)	131.57
3′	6.92 (d, 8.7)	115.79	6.92 (d, 9)	116.05
4′	-	162.47	-	162.87
5′	6.92 (d, 8.7)	115.79	6.92 (d, 9)	116.05
6′	7.95 (d, 8.7)	131.27	7.95 (d, 9)	131.57
C=O	-	199.99	-	200.37
α	3.16 (t, 7.1)	38.95	3.15 (t, 7)	39.02
β	2.90 (t, 7.1)	18.55	2.90 (t, 7)	18.57
OCH_3-4	3.71 (s)	55.26	3.72 (s)	55.38
OCH_3-6	3.78 (s)	55.76	3.79 (s)	55.87

1.2 Identification of Compound DL-B

Compound DL-B was obtained as colorless needles. The UV spectrum (Figure 10) showed maximal absorptions at 218, 238 (sh), 306 and 319 nm. Its IR spectrum (Figure 11) exhibited absorption bands at 3415 (OH), 1596 (>C=C<), 1514 (aromatic ring), 1155 (ether linkage) and 961 (*trans*-CH=CH-) cm^{-1}. The EI mass

spectrum (Figure 12) displayed a molecular ion peak at *m/z* 256, suggesting the molecular formula $C_{16}H_{16}O_3$. Compound DL-B was identified as pterostilbene [**51**]. The ^{1}H-NMR spectrum (Figure 13) of compound DL-B exhibited signals for two methoxyl groups at δ 3.82. The singlet signal at δ 6.37 was assigned to the H-4′ and the 2H doublet signal (J = 2.1 Hz) at δ 6.64 was assigned to H-2′ and H-6′. The doublet signals (J = 16.4 Hz) at δ 6.87 and 7.01 were assigned to the *trans* olefinic protons H-β and H-α. The *ortho*-coupled doublets (J = 8.6 Hz) at δ 6.81 (2H, H-3 and H-5) and δ 7.38 (2H, H-2 and H-6) revealed the presence of *p*-substituted benzene ring, and this was confirmed by the correlation peak in the ^{1}H-^{1}H COSY spectrum (Figure 14). Pterostilbene has been isolated from several species of *Pterocarpus* (King *et al*., 1953), *Pterolobium hexapetalum* (Kumar *et al*., 1988) and *Dracaena cochinchinensis* (Lu *et al*., 1988; Wei *et al*., 1988), but its ^{1}H and ^{13}C NMR data have never been reported. In the present investigation, complete ^{1}H and ^{13}C-NMR assignments were therefore obtained by analysis of the DEPT (Figure 15), HETCOR (Figures 16a and 16b) and HMBC (Figure 17) spectra, as summarized in Table 10. This study provides the first ^{1}H and ^{13}C NMR reports for this compound.



[**51**]

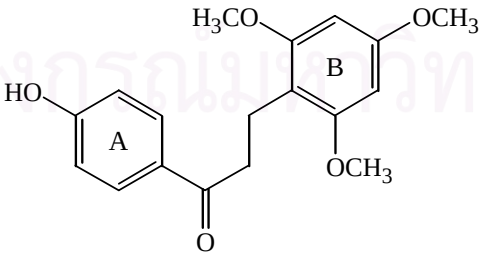
Table 10 ^{1}H and ^{13}C NMR, and HMBC spectral data of compound DL-B (in $CDCl_3$)

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC (Correlation with carbon)
1	-	129.95	-
2	7.38 (d, 8.6)	127.87	C-4, C-6 and C-α
3	6.81 (d, 8.6)	115.54	C-1 and C-4*
4	-	155.23	-
5	6.81 (d, 8.6)	115.54	C-1 and C-4*
6	7.38 (d, 8.6)	127.87	C-2, C-4 and C-α
1′	-	139.53	-
2′	6.64 (d, 2.1)	104.36	C-3′*, C-4′ and C-6′
3′	-	160.72	-
4′	6.37 (s)	99.61	C-2′ and C-6′
5′	-	160.72	-
6′	6.64 (d, 2.1)	104.36	C-2′, C-4′ and C-5′*
α	7.01 (d, 16.4)	128.59	C-2, C-6 and C-1′
β	6.87 (d, 16.4)	126.46	C-1, C-1′*, C-2′, C-6′ and C-α*
OCH_3-3′	3.82 (s)	55.42	C-3′
OCH_3-5′	3.82 (s)	55.42	C-5′

*Two-bond coupling

1.3 Structure Determination of Compound DL-C

Compound DL-C showed UV absorptions (Figure 18) at 207, 223 (sh) and 277 nm. The IR spectrum of compound DL-C (Figure 19) exhibited absorption bands for OH stretching at 3423 cm^{-1} (broad peak), C=O at 1649 cm^{-1}, aromatic ring at 1605-1501 cm^{-1} and ether linkage at 1180 cm^{-1}. The EI mass spectrum (Figure 20) revealed $[M^+]$ at *m/z* 316, corresponding to the molecular formula $C_{18}H_{20}O_5$, and a peak at *m/z* 121 $[M\text{-}C_{11}H_{15}O_3]^+$. The difference in the molecular weights of DL-A and DL-C indicated that DL-C had three methoxyl groups. DL-C was identified as loureirin B [**53**] by comparison of its ^1H and ^{13}C NMR data with reported values (Meksuriyen and Cordell, 1988a). The ^1H-NMR spectrum (Figure 21) showed two multiplet signals at δ 2.89 and δ 2.98 due to H-β and H-α. The three methoxyl groups appeared as one singlet signal (9H) at δ 3.79, and the singlet signal at δ 6.23 (2H) was assigned to H-3 and H-5. The *ortho*-coupled doublets (J = 8.6 Hz) at δ 6.92 (2H, H-3′ and H-5′) and δ 7.92 (2H, H-2′ and H-6′) revealed *p*-substitution of ring A. The ^{13}C-NMR and DEPT spectra (Figure 22) showed the presence of three methyl, two methylene, six methine and seven quaternary carbons. The ^1H and ^{13}C NMR of DL-C are in excellent agreement with the previous data (Meksuriyen and Cordell, 1988a) (Table 11).



[**53**]

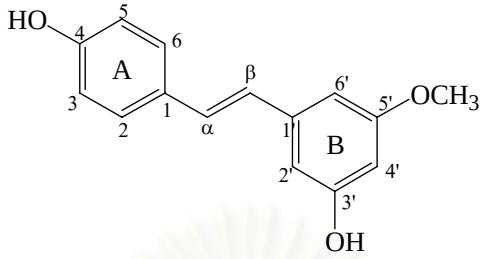
Table 11 ^{1}H and ^{13}C NMR spectral data of compound DL-C (in acetone-d_6) and loureirin B (in $CDCL_3$)

Position	Compound DL-C		Loureirin B	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	109.96	-	109.51
2	-	160.43	-	159.50
3	6.23 (s)	91.28	6.11 (s)	90.40
4	-	159.29	-	158.63
5	6.23 (s)	91.28	6.11 (s)	90.40
6	-	160.43	-	159.50
1′	-	129.89	-	129.15
2′	7.92 (d, 8.6)	131.05	7.93 (d, 8.7)	130.03
3′	6.92 (d, 8.6)	115.71	6.93 (d, 8.7)	115.40
4′	-	162.10	-	161.26
5′	6.92 (d, 8.6)	115.71	6.93 (d, 8.7)	115.40
6′	7.92 (d, 8.6)	131.05	7.93 (d, 8.7)	130.03
C=O	-	198.40	-	201.48
α	2.98 (m)	38.88	3.07 (m)	38.48
β	2.89 (m)	19.37	2.99 (m)	18.90
OCH_3-2	3.79 (s)	55.87	3.77 (s)	55.52
OCH_3-4	3.79 (s)	55.45	3.78 (s)	55.31
OCH_3-6	3.79 (s)	55.87	3.77 (s)	55.52

1.4 Structure Determination of Compound DL-D

Compound DL-D showed UV absorptions (Figure 23) at 217, 238 (sh), 305 and 318 nm. The IR spectrum (Figure 24) exhibited absorption bands for hydroxyl groups at 3438 cm^{-1}, C=C stretching at 1606 cm^{-1}, aromatic ring at 1514 cm^{-1}, ether linkage at 1153 cm^{-1} and *trans*-CH=CH- at 961 cm^{-1}. The EIMS of compound DL-D (Figure 25) revealed a molecular ion peak at *m/z* 242, corresponding to the molecular formula $C_{15}H_{14}O_3$. The difference in the molecular weights of DL-B and DL-D indicated that DL-D had only one methoxyl group. The ^{1}H-NMR spectrum (Figure 26) showed a methoxyl signal at δ 3.76. The doublet signal (J = 1.5 Hz) at δ 6.63 could be assigned to H-2′ and H-6′. The peak of *meta*-coupled triplet (J = 1.5 Hz) at δ 6.31 was assigned to H-4′. The *ortho*-coupled doublets (J = 8.7 Hz) at δ 6.84 (2H, H-3 and H-5) and δ 7.42 (2H, H-2 and H-6) revealed the presence of *p*-substituted benzene ring. A pair of doublet signals (J = 16.4 Hz) at δ 6.92 and δ 7.09 were due to the *trans* olefinic protons H-β and H-α, and this was confirmed by the correlation peak in the NOESY. From the NOESY spectrum (Figures 27a and 27b), the H-2 and H-6 (δ 7.42) signals showed NOE interactions with the resonances of H-α (δ 7.09), H-β (δ 6.92). H-6′ (δ 6.63) showed NOE interaction with the methoxyl group (δ 3.76) and H-β (δ 6.92). The methoxyl group (δ 3.76) showed NOE interaction with H-4′ (δ 6.31) and H-6′ (δ 6.63). The ^{13}C-NMR, DEPT and HETCOR spectra (Figures 28-29) exhibited signals for one methyl carbon, nine methine and five quaternary carbons. The complete ^{13}C NMR assignments of compound DL-D were obtained from the HMBC correlations (Figures 30a and 30b). The H-C long-range correlations from the HMBC spectrum of compound DL-D are summarized in Table 12. From all of the above spectral data, compound DL-D was identified as pinostilbene [**145**]. This compound has been isolated from the *Pinus sibilica*

(Tyukavkina *et al*., 1972). The present study provides the first ^{1}H and ^{13}C NMR reports for this compound.



[**145**]

Table 12 ^{1}H and ^{13}C NMR, and HMBC spectral data of compound DL-D (in acetone-d_6)

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC (correlation with carbon)
1	-	130.09	-
2	7.42 (d, 8.7)	128.97	C-4, C-6 and C-α
3	6.84 (d, 8.7)	116.68	C-1, C-5 and C-4*
4	-	158.38	-
5	6.84 (d, 8.7)	116.68	C-1, C-3 and C-4*
6	7.42 (d, 8.7)	128.97	C-2, C-4 and C-α
1′	-	141.08	-
2′	6.63 (d, 1.5)	106.98	C-4′, C-6′ and C-β
3′	-	159.69	-
4′	6.31 (t, 1.5)	101.64	C-2′ and C-6′

*Two-bond coupling

Table 12 ^{1}H and ^{13}C NMR and HMBC spectral data of compound DL-D (in acetone-d_6) (continued)

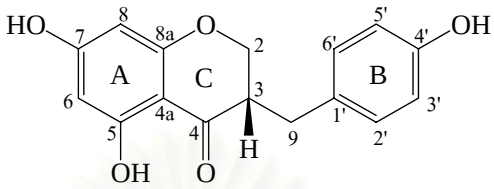
Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC (correlation with carbon)
5′	-	162.27	-
6′	6.63 (d, 1.5)	104.27	C-2′, C-4′ and C-β
α	7.09 (d, 16.4)	129.67	C-2, C-6, C-1′ and C-β*
β	6.92 (d, 16.4)	126.91	C-1, C-2′, C-6′ and C-α*
OCH_3-5′	3.76 (s)	55.85	C-5′

*Two-bond coupling

1.5 Structure Determination of Compound DL-E

Compound DL-E was obtained as pink needles. It showed UV absorptions at 212, 225 (sh), 289 and 328 nm (Figure 31). The IR spectrum (Figure 32) exhibited a hydroxyl group at 3429, an aromatic ring at 1515, a chelated carbonyl functionality at 1643 and an ether linkage at 1161 cm^{-1}. The EIMS of compound DL-E (Figure 33) revealed a molecular ion peak at *m/z* 286, corresponding to the molecular formula $C_{16}H_{14}O_5$. It showed the base peak at 107 $[M-C_9H_7O_4]^+$. By analyses of the ^{1}H and ^{13}C NMR data and comparison with previously reported data (Adinolfi *et al*., 1985), compound DL-E was identified as 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**83**]. The ^{1}H-NMR spectral data (Figures 34a and 34b) showed signals for H_2-9 at δ 2.66 and δ 3.12, H-3 at δ 2.90 and H_2-2 at δ 4.12 and δ 4.30. The doublet signals (*J* = 2.1 Hz) at δ 5.90 and δ 5.93 were assigned to the *meta* coupled protons H-6 and H-8. The *ortho*-coupled doublets (*J* = 8.2 Hz) at δ 6.79 (2H, H-3′ and H-5′) and δ 7.10 (2H, H-2′ and H-6′) indicated the presence of *p*-substituted benzene ring. The

^{13}C-NMR and DEPT spectra (Figure 35) showed the presence of two methylene, seven methine and seven quaternary carbons. Table 13 compares ^{1}H and ^{13}C NMR data of DL-E with reported values.



[**83**]

Table 13 ^{1}H and ^{13}C NMR spectral data of compound DL-E (in acetone-d_6) and 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone (in DMSO-d_6)

Position	Compound DL-E		5,7-Dihydroxy-3-(4-hydroxybenzyl)-4-chromanone	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
2	4.12 and 4.30 (dd, 11.4, 11.4)	69.82	4.10 and 4.28 (m) AB of ABX	68.8
3	2.90 (m)	47.20	2.97 (m)	45.5
4	-	198.42	-	197.7
4a	-	102.48	-	101.0
5	-	165.23	-	163.6
6	5.90 (d, 2.1)	96.70	5.87 (br s)	95.9
7	-	166.94	-	166.7
8	5.93 (d, 2.1)	95.36	5.87 (br s)	94.7
8a	-	163.89	-	162.6

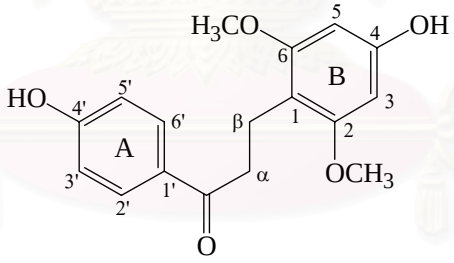
Table 13 ^{1}H and ^{13}C NMR spectral data of compound DL-E (in acetone-d_6) and 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone (in DMSO-d_6) (continued)

Position	Compound DL-E		5,7-Dihydroxy-3-(4-hydroxybenzyl)-4-chromanone	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
9	2.66 and 3.12 (dd, 13.8, 13.8)	32.24	2.63 and 3.05 (m)	31.2
1′	-	129.47	-	128.0
2′	7.10 (d, 8.2)	130.59	7.07 (d, 7.93)	129.8
3′	6.79 (d, 8.2)	115.98	6.74 (d, 7.93)	115.1
4′	-	156.64	-	155.5
5′	6.79 (d, 8.2)	115.98	6.74 (d, 7.93)	115.1
6′	7.10 (d, 8.2)	130.59	7.07 (d, 7.93)	129.8
OH	12.22 (br s)	-	12.24 (s)	-

1.6 Structure Determination of Compound DL-F

Compound DL-F was obtained as an orange amorphous powder. The UV spectrum (Figure 36) showed maximal absorptions at 209, 223 (sh) and 278 nm. The IR spectrum (Figure 37) exhibited absorption bands at 3415 (OH), 1661 (C=O), 1604 (>C=C<), 1511 (aromatic ring) and 1163 (C-O stretching of ether) cm^{-1}. The EI mass spectrum (Figure 38) displayed a molecular ion peak at *m/z* 302, suggesting the molecular formula $C_{17}H_{18}O_5$. Other major peaks appeared at *m/z* 167 $[M\text{-}C_8H_7O_2]^+$ and 121 $[M\text{-}C_{10}H_{13}O_3]^+$. DL-F had the same molecular weight as DL-A. Compound

DL-F was identified as 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone [**100**] (Ichikawa *et al*., 1997). The ^{1}H-NMR spectrum (Figure 39) of compound DL-F exhibited six proton signals in the aromatic proton regions. The multiplet aliphatic proton signals at δ 2.86 and δ 2.95 were assigned to the methylene protons H-*β* and H-*α*. The *ortho*-coupled doublets (*J* = 8.6 Hz) at δ 6.91 (2H, H-3′ and H-5′) and δ 7.91(2H, H-2′ and H-6′) revealed *p*-substitution for ring A. The two methoxyl groups at δ 3.73 (6H, s) were different from those of DL-A. The ^{13}C-NMR and DEPT spectra (Figure 40) showed the presence of two methyl, two methylene, six methine and seven quaternary carbons. This compound was different from DL-A because its C-2 and C-6 showed at the similar chemical shift so as to its C-3 and C-5. The ^{1}H and ^{13}C NMR data of DL-F were compared with those of 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone as summarized in Table 14.



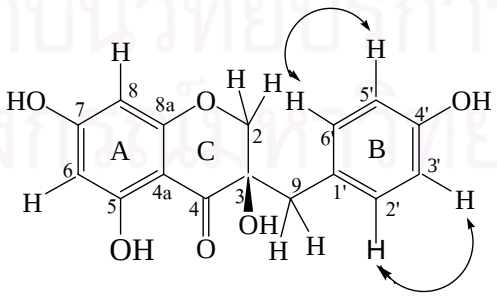
[**100**]

Table 14 ^{1}H and ^{13}C NMR spectral data of compound DL-F (in acetone-d_6) and 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone (in acetone-d_6)

Position	Compound DL-F		4,4′-Dihydroxy-2,6-dimethoxydihydrochalcone	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	108.73	-	108.91
2	-	159.35	-	158.15
3	6.13 (s)	92.54	6.14 (s)	92.68
4	-	157.81	-	159.70
5	6.13 (s)	92.54	6.14 (s)	92.68
6	-	159.35	-	158.15
1′	-	129.91	-	130.19
2′	7.91 (d, 8.6)	131.07	7.95 (d, 9)	131.57
3′	6.91 (d, 8.6)	115.74	6.92 (d, 9)	115.95
4′	-	162.11	-	162.42
5′	6.91 (d, 8.6)	115.74	6.92 (d, 9)	115.95
6′	7.91 (d, 8.6)	131.07	7.95 (d, 9)	131.57
C=O	-	198.54	-	198.88
α	2.95 (m)	39.05	2.91 (m)	39.09
β	2.86 (m)	19.36	2.91 (m)	19.32
OCH_3-2	3.73 (s)	55.74	3.75 (s)	55.81
OCH_3-6	3.73 (s)	55.74	3.75 (s)	55.81

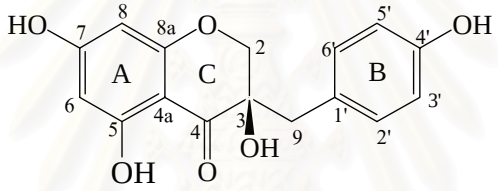
1.7 Structure Determination of Compound DL-G

Compound DL-G was obtained as a yellowish amorphous powder. The UV spectrum (Figure 41) showed maximal absorptions at 214, 224 (sh), 292 and 330 nm. The IR spectrum (Figure 42) exhibited absorption bands at 3416 (OH), 1643 (C=O), 1593-1515 (aromatic ring) and 1163 (C-O stretching ether) cm^{-1}. The HREIMS (Figure 43) revealed a molecular ion at *m/z* 302.07997 (calcd. 302.07904), corresponding to the molecular formula $C_{16}H_{14}O_6$. The ^{1}H-NMR spectrum (Figure 44) of DL-G showed close similarity to that of (3*R*)-Eucomol [**99**] (Meksuriyen and Cordell, 1988b) except for the absence of the signal for the methoxyl group. Signals for two *ortho*-coupled doublets (J = 8.4 Hz) appeared at δ 6.77 (2H, H-3′ and H-5′) and δ 7.11 (2H, H-2′ and H-6′). The methylene protons H_2-9 appeared as two doublet signals at δ 2.88 (1H, d, J = 14.1 Hz) and δ 2.95 (1H, d, J = 14.1 Hz), and H_2-2 exhibited at δ 4.02 (1H, d, J = 11.4 Hz) and δ 4.10 (1H, d, J = 11.4 Hz). The other peaks were found at δ 5.99 (2H, d, J = 3.0 Hz, H-6 and H-8) and δ 11.77 (1H, br s, OH). ^{1}H-^{1}H COSY and NOESY experiments were performed (Figures 45 and 46). From the NOESY spectrum, the H-2′ and H-6′ (δ 7.11) proton showed NOE interactions with the resonances of H-3′ and H-5′ (δ 6.77).



The ^{13}C-NMR and DEPT spectra (Figure 47) showed the presence of two methylene, six methine and eight quaternary carbons. HETCOR and COLOC

experiments were carried out to establish the structure (Figures 48-49). The quaternary carbons at C-4a and C-5 positions were correlated to the proton at H-6; C-3 was correlated to H_2-2 and H_2-9; C-7 was correlated to H-6 and H-8; C-1′ was correlated to H-3′, H-5′ and H-9; C-4′ was correlated to H-2′ and H-6′; and C-8a was correlated to H_2-2 and H-8. Thus, DL-G was identified as 3,5,7-trihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**146**] (Table 15). The sign of the optical rotation of DL-G $[\alpha]^{20}_{D}$ was negative, similar to that of (3*R*)-Eucomol [**99**] (Meksuriyen and Cordell, 1988b). Therefore, it was concluded that DL-G had 3R configuration. DL-G is a new homoisoflavanone.



[**146**]

Table 15 ^{1}H and ^{13}C NMR, and COLOC spectral data of compound DL-G (in acetone-d_6)

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	COLOC (correlation with proton)
2	4.02 (d, 11.4) and 4.10 (d, 11.4)	72.49	H_2-9
3	-	72.92	H_2-2* and H_2-9*
4	-	199.03	H_2-2 and H_2-9
4a	-	100.89	H-6 and H-8

*Two-bond coupling

Table 15 ^{1}H and ^{13}C NMR, and COLOC spectral data of compound DL-G (in acetone-d_6) (continued)

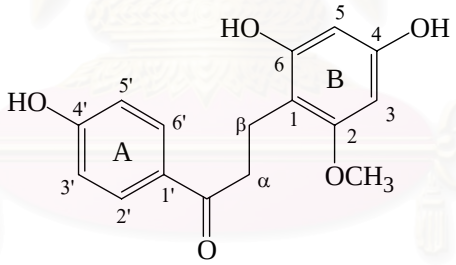
Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	COLOC (correlation with proton)
5	-	165.10	H-6*
6	5.99 (d, 3.0)	95.76	H-8
7	-	167.46	H-6* and H-8*
8	5.99 (d, 3.0)	97.04	H-6
8a	-	163.65	H_2-2 and H-8*
9	2.88 (d, 14.1) and 2.95 (d, 14.1)	40.46	H-2′ and H-6′
1′	-	126.24	H_2-9*, H-3′ and H-5′
2′	7.11 (d, 8.4)	132.27	H_2-9, H-3′* and H-6′
3′	6.77 (d, 8.4)	115.45	H-2′* and H-5′
4′	-	156.88	H-2′ and H-6′
5′	6.77 (d, 8.4)	115.45	H-3′ and H-6′*
6′	7.11 (d, 8.4)	132.27	H_2-9, H-2′ and H-5′*
OH	11.77 (br s)	-	-

*Two-bond coupling

1.8 Structure Determination of Compound DL-H

The UV spectrum of DL-H (Figure 50) showed maximal absorptions at 207, 223 (sh) and 279 nm. The IR spectrum (Figure 51) of compound DL-H exhibited absorption bands for hydroxyl groups at 3414 cm^{-1}, a carbonyl group at 1654 cm^{-1}, aromatic rings at 1473 and 1600 cm^{-1}, and an ether linkage at 1169 cm^{-1}. Its EIMS

(Figure 52) revealed a molecular ion at m/z 288, suggesting the molecular formula $C_{16}H_{16}O_5$. Other major peaks were found at 153 $[M\text{-}C_8H_7O_2]^+$, 140 $[M\text{-}C_9H_8O_2]^+$ and 121 $[M\text{-}C_9H_{11}O_3]^+$. The ^{1}H-NMR spectrum (Figure 53) of compound DL-H exhibited two triplet signals (J = 7.2 Hz) at δ 2.87 and δ 3.12 assignable to the methylene protons H-β and H-α. The methoxyl signal appeared at δ 3.73. The *meta* coupled protons at δ 6.02 and δ 6.06 were assigned to H-3 and H-5 (J = 2.1 Hz). The *ortho*-coupled doublets (J = 8.7 Hz) at δ 6.91 (d, H-3′ and H-5′) and δ 7.94 (d, H-2′ and H-6′) revealed the presence of a *p*-substituted benzene ring. The ^{13}C-NMR and DEPT spectral data (Figure 54) provided signals for one methyl, two methylenes, six methines and seven quaternary carbons. Compound DL-H was identified as loureirin D [**94**] (Meksuriyen and Cordell, 1988a). Table 16 summarizes its NMR properties as compared with reported values.



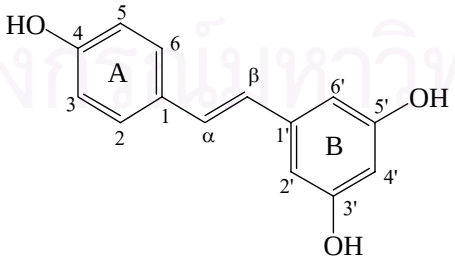
[**94**]

Table 16 ^{1}H and ^{13}C NMR spectral data of compound DL-H (in acetone-d_6) and loureirin D (in CD_3OD)

Position	Compound DL-H		Loureirin D	
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	107.78	-	108.11
2	-	159.80	-	160.34
3	6.02 (d, 2.1)	91.87	5.99 (d, 2)	91.87
4	-	157.56	-	157.72
5	6.06 (d, 2.1)	96.65	6.03 (d, 2)	96.48
6	-	156.87	-	157.28
1′	-	129.66	-	129.68
2′	7.94 (d, 8.7)	131.25	7.90 (d, 8.7)	132.01
3′	6.91 (d, 8.7)	115.78	6.84 (d, 8.7)	116.05
4′	-	162.43	-	163.51
5′	6.91 (d, 8.7)	115.78	6.84 (d, 8.7)	116.05
6′	7.94 (d, 8.7)	131.25	7.90 (d, 8.7)	132.01
C=O	-	199.97	-	202.86
α	3.12 (t, 7.2)	39.10	3.08 (t, 7.4)	39.47
β	2.87 (t, 7.2)	18.63	2.91 (t, 7.4)	19.79
OCH_3-2	3.73 (s)	55.65	3.70 (s)	55.73
OH	8.50 (br s)	-	-	-

1.9 Structure Determination of Compound DL-I

The UV spectrum of DL-I (Figure 55) showed maximal absorptions at 217, 237 (sh), 304 and 318 nm. The IR spectrum (Figure 56) exhibited absorption bands at 3306 (OH), 1607 (>C=C<), 1514 (aromatic ring) and 966 (*trans*-CH=CH-) cm^{-1}. The EI mass spectrum (Figure 57) displayed a molecular ion peak at *m/z* 228, suggesting the molecular formula $C_{14}H_{12}O_3$. The ^{1}H-NMR spectrum of compound DL-I (Figure 58) exhibited a pair of doublet signals (J = 16.5 Hz) at δ 6.88 and δ 7.02 assignable to the *trans* olefinic protons H-β and H-α. The *ortho*-coupled doublets (J = 8.7 Hz) at δ 6.83 (2H, H-3 and H-5) and δ 7.42 (2H, H-2 and H-6) revealed the presence of a *p*-substituted benzene ring. The other proton signals at δ 6.27 (H-4′, t, J = 1.8 Hz), δ 6.54 (H-2′ and H-6′, d, J = 1.8 Hz) revealed the presence of *meta*-substitution on ring B. The ^{13}C-NMR and DEPT spectra (Figure 59) provided signals for nine methine and five quaternary carbons. The direct correlation of H and C atoms was studied by examination of the HETCOR spectrum (Figure 60). The ^{1}H and ^{13}C NMR spectra of DL-I showed close similarity to those of compounds DL-B and DL-D except for the absence of a methoxyl signal. By comparing the above spectral information with reported ^{1}H and ^{13}C NMR data (Sritulalak, 1998), compound DL-I was identified as resveratrol [**43**] (Table 17).



[**43**]

Table 17 ^{1}H and ^{13}C NMR spectral data of compound DL-I (in acetone-d_6) and resveratrol (in DMSO-d_6)

Position	Compound DL-I		Resveratrol	
	δ_H (ppm) (multioplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)
1	-	129.69	-	127.8
2	7.42 (d, 8.7)	128.43	7.39 (d, 8.4)	127.6
3	6.83 (d, 8.7)	116.15	6.75 (d, 8.4)	115.4
4	-	157.78	-	156.9
5	6.83 (d, 8.7)	116.15	6.75 (d, 8.4)	115.4
6	7.42 (d, 8.7)	128.43	7.39 (d, 8.4)	127.6
1′	-	140.57	-	139.0
2′	6.54 (d, 1.8)	105.48	6.38 (d, 1.8)	104.1
3′	-	159.20	-	158.2
4′	6.27 (t, 1.8)	102.44	6.11 (s)	101.6
5′	-	159.20	-	158.2
6′	6.54 (d, 1.8)	105.48	6.38 (d, 1.8)	104.1
α	7.02 (d, 16.5)	128.82	6.39 (d, 16.5)	127.6
β	6.88 (d, 16.5)	126.56	6.81 (d, 16.5)	125.4
OH	8.28 (br s)	-	-	-

2. **Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) Inhibitory Activity of Pure Compounds**

In this study, evaluations of the COX-2 and COX-1 inhibitory activity of pure compounds and crude extracts were performed using Kirtikara's method (Kirtikara *et al*., 2001). The ethyl acetate and methanol extracts of *D. loureiri* showed 97 % inhibition of COX-2 at 100 μg/ml. Pure compounds were first tested at 10 μg/ml. Compounds exhibiting more than 80% inhibition were further analyzed for their IC_{50} values. Aspirin [**147**], NS-398 [**148**] and indomethacin [**149**] were used as positive controls. The results are summarized in Table 8.

It can be seen from Table 8 that the stilbene derivatives, pterostilbene [**51**], pinostilbene [**145**] and resveratrol [**43**] were potent inhibitors of both COX enzymes (IC_{50} 1.29-4.92 μM), being more active than aspirin (IC_{50} 11.41-19.80 μM). This may imply the role of these stilbenes in the fever- and pain-relieving effects of *D. loureiri*. However, the COX inhibitory activities of these stilbenes were less than those of indomethacin (IC_{50} 0.005-0.006 μM). It should be noted that no selectivity was observed for these natural compounds, as compared with NS-398, a well-known selective COX-2 inhibitor. The flavonoid compounds showed weak or no activity. The homoisoflavanone derivative, 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**83**] and the retrodihydrochalcones: 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone [**101**], 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone [**100**] and loureirin D [**94**] showed the weak activity. Loureirin B [**53**] and 3,5,7-trihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**146**] were inactive. It should be pointed out that the biological data in this study provide supporting evidence for the medicinal use of *D. loureiri*.

CHAPTER V

CONCLUSION

Nine pure compounds were isolated from the stem of *Dracaena loureiri* Gagnep. These include 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone [**101**], loureirin B [**53**], 4,4′-dihydroxy-2,6-dimethoxydihydrochalcone [**100**], loureirin D [**94**], 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**83**], pterostilbene [**51**], pinostilbene [**145**], resveratrol [**43**] and a new homoisoflavanone named 3,5,7-trihydroxy-3-(4-hydroxybenzyl)-4-chromanone [**146**]. The unambiguous ^{1}H and ^{13}C NMR assignments of pterostilbene [**51**] and pinostilbene [**145**] were also obtained for the first time in this study. The stilbenes pterostilbene [**51**], pinostilbene [**145**] and resveratrol [**43**] showed potent COX-2 inhibition (IC_{50} values of 1.29, 2.21 and 2.16 μM, respectively) and strong COX-1 inhibition (IC_{50} values of 4.84, 4.92 and 2.61 μM, respectively). The study exemplifies an attempt to find scientific evidence to support the traditional medicinal claims. It also provides useful information on the phytochemistry and chemotaxonomy of the genus *Dracaena*.

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APPENDIX

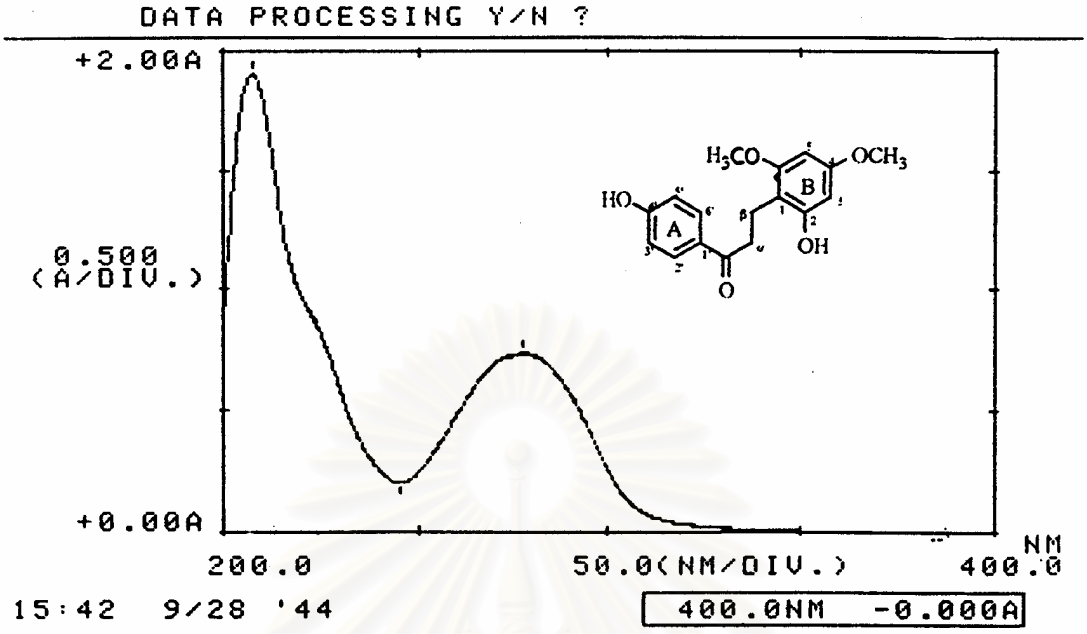

Figure 5 UV spectrum of compound DL-A (in methanol)

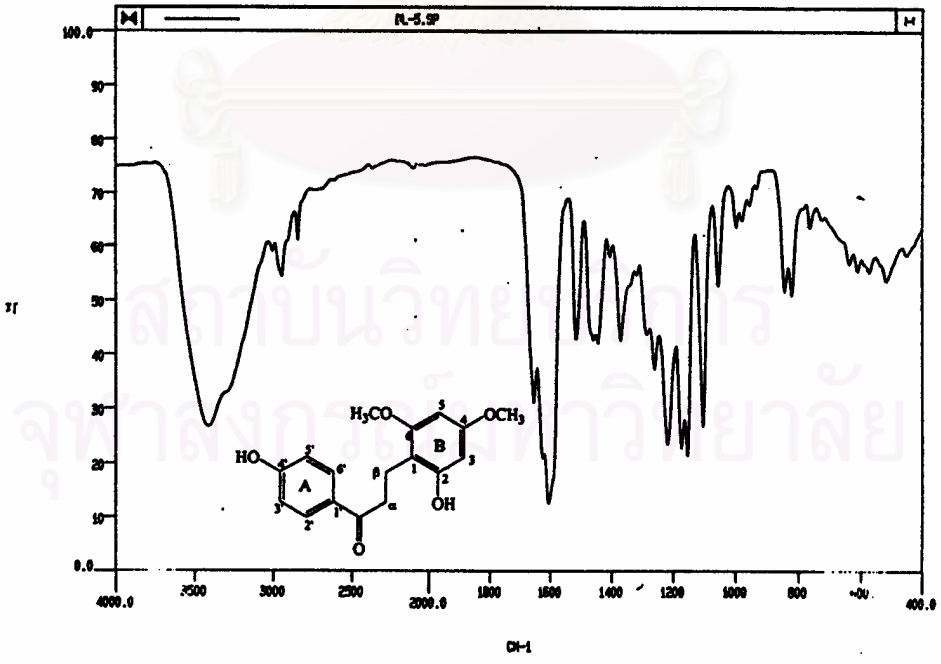

Figure 6 IR spectrum of compound DL-A (KBr disc)

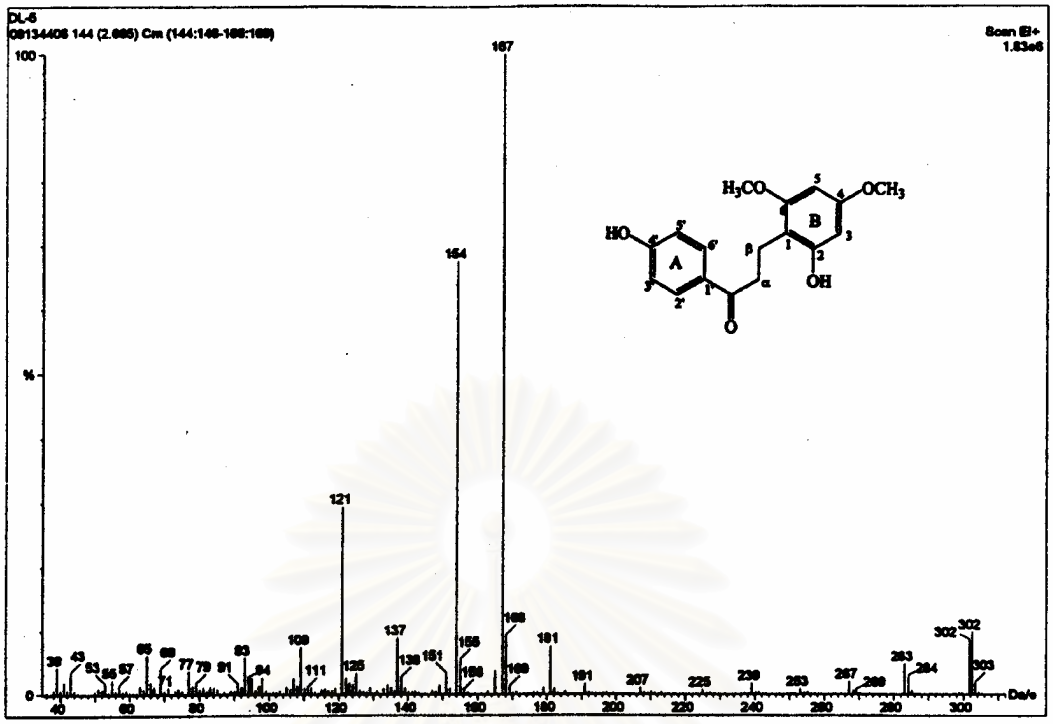

Figure 7 EI mass spectrum of compound DL-A

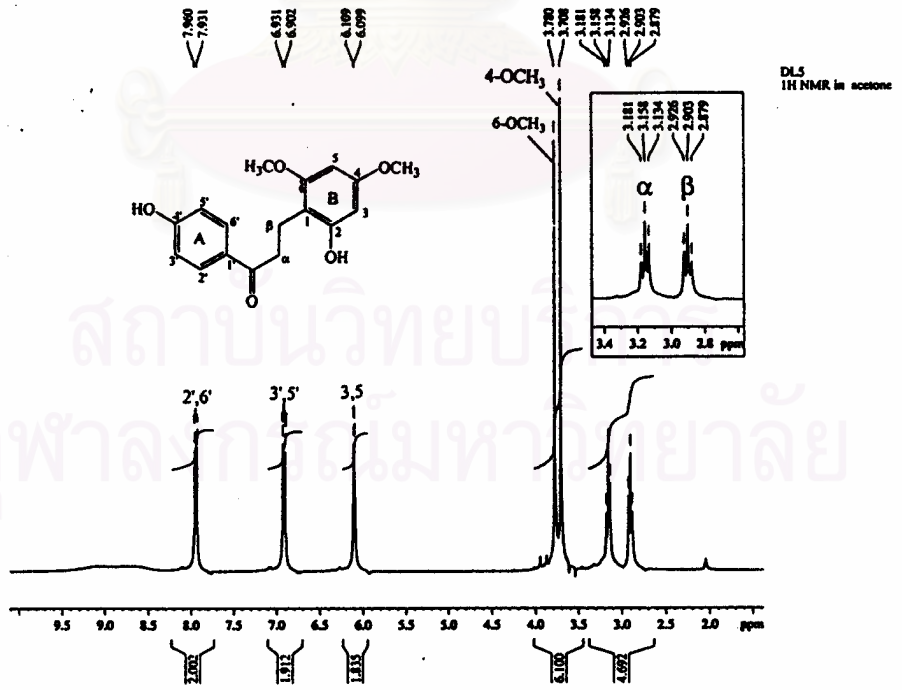

Figure 8 300 MHz ^{1}H NMR spectrum of compound DL-A (in acetone-d_6)

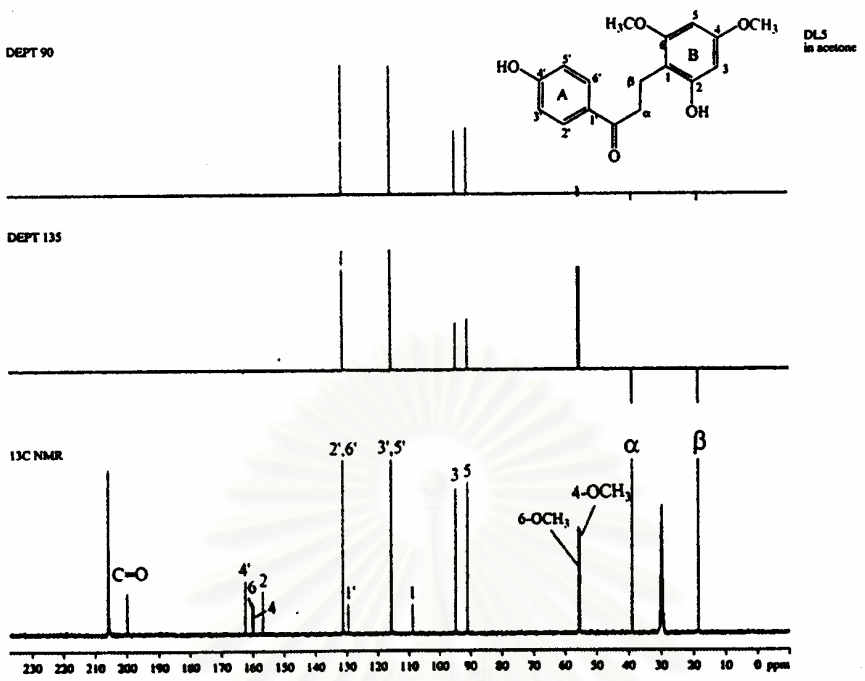

Figure 9 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-A (in acetone-d_6)

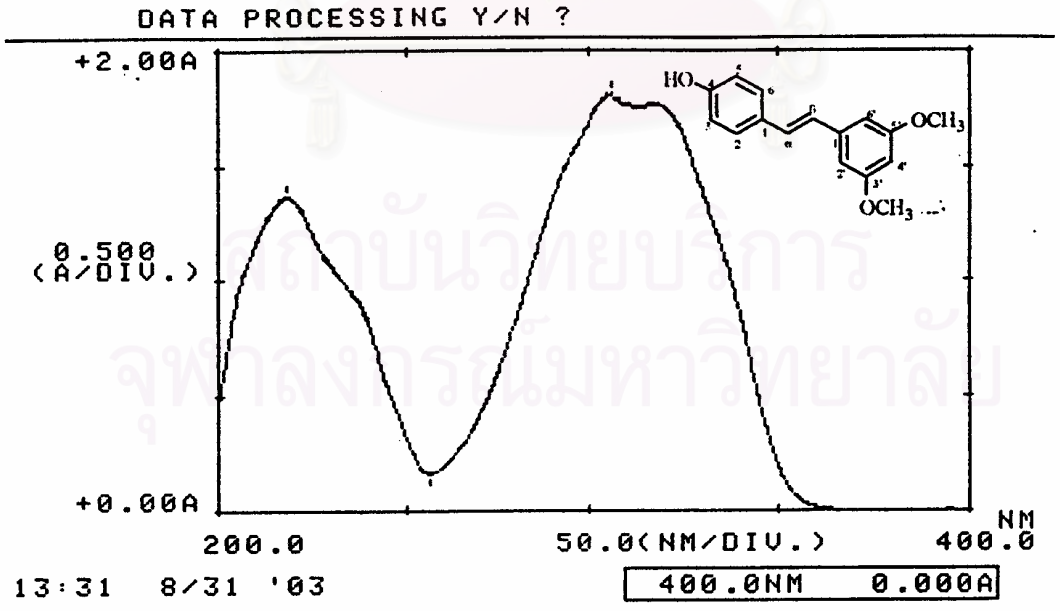

Figure 10 UV spectrum of compound DL-B (in methanol)

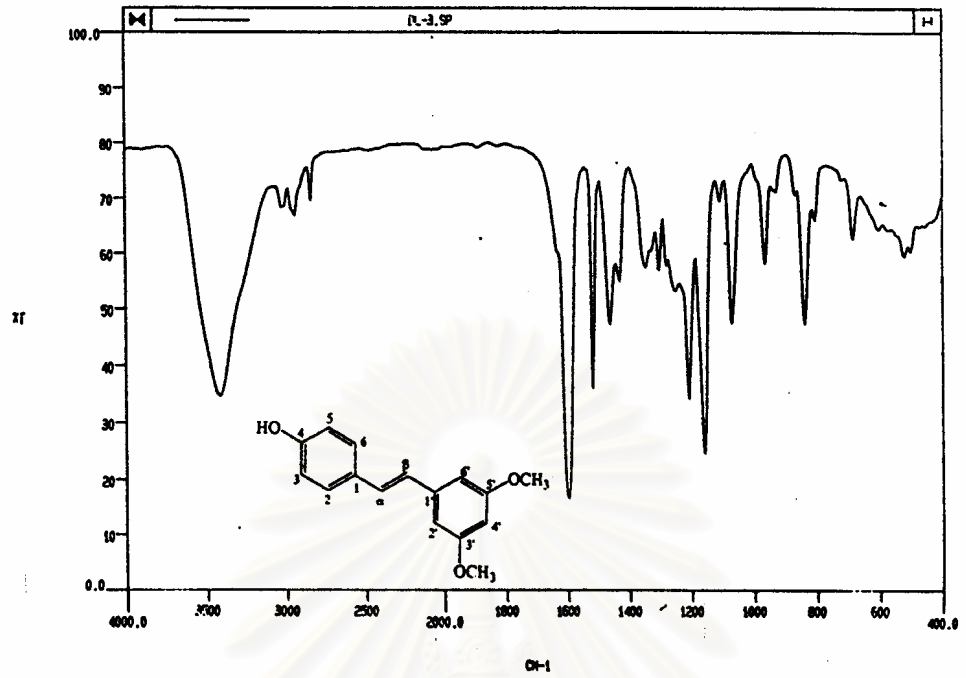

Figure 11 IR spectrum of compound DL-B (KBr disc)

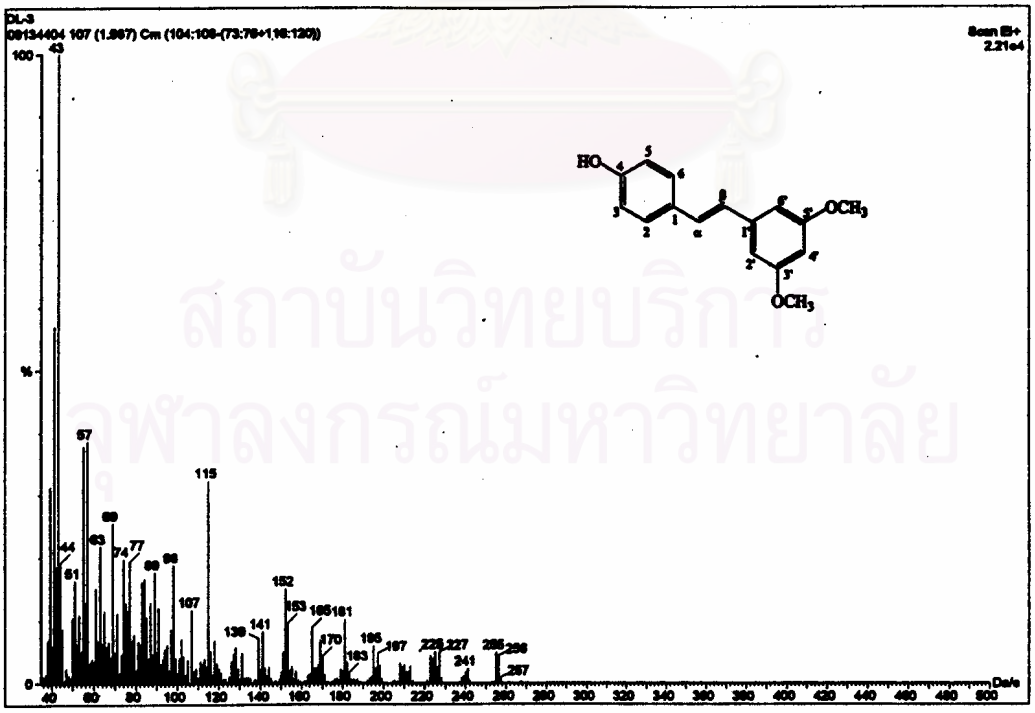

Figure 12 EI mass spectrum of compound DL-B

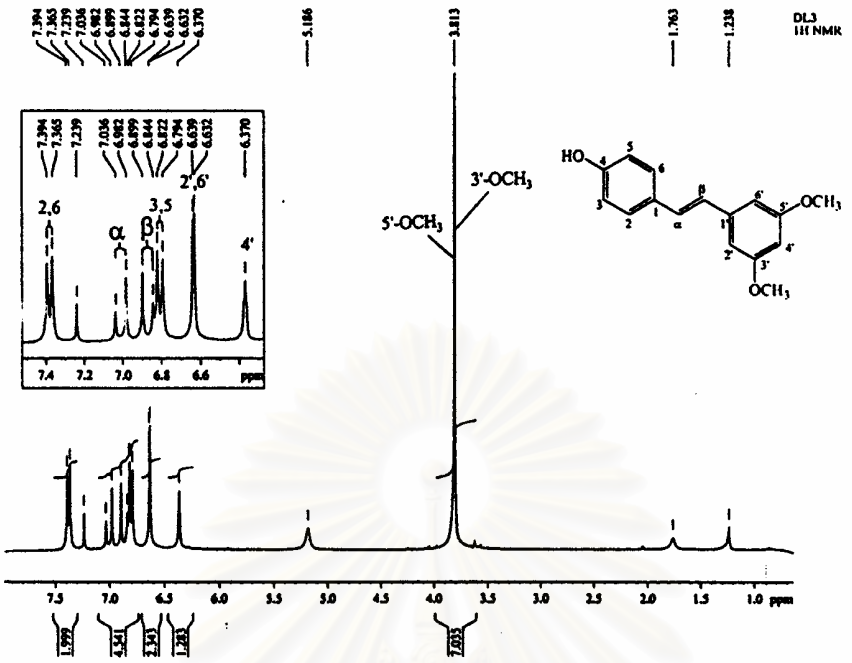

Figure 13 300 MHz ^{1}H NMR spectrum of compound DL-B (in $CDCl_3$)

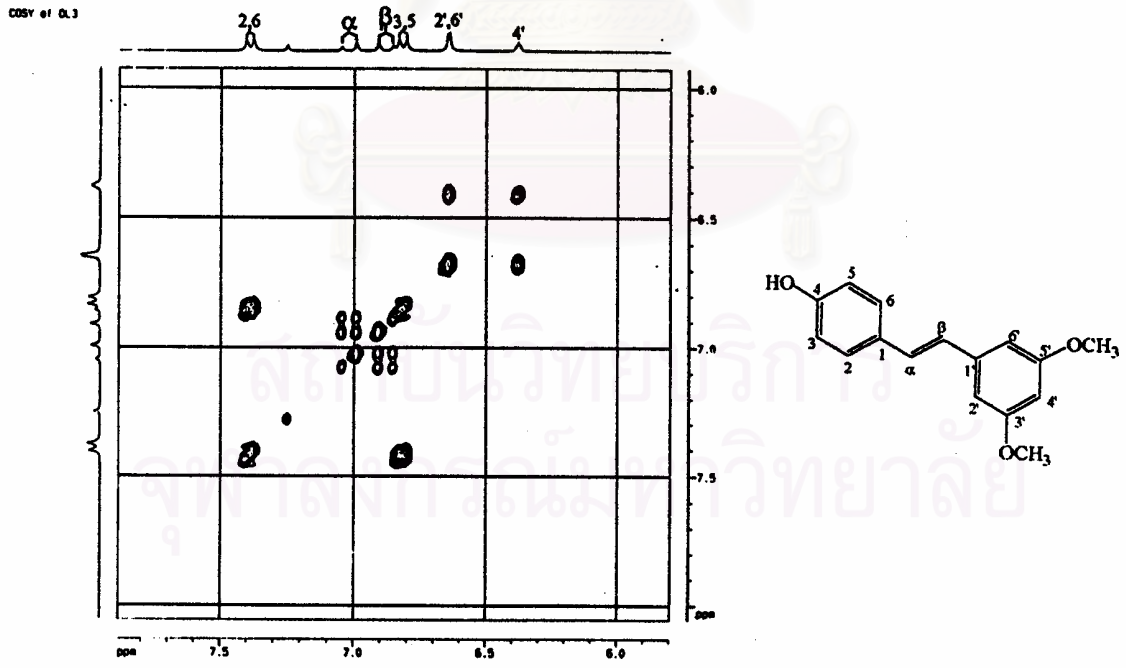

Figure 14 ^{1}H-^{1}H COSY spectrum of compound DL-B (in $CDCl_3$)

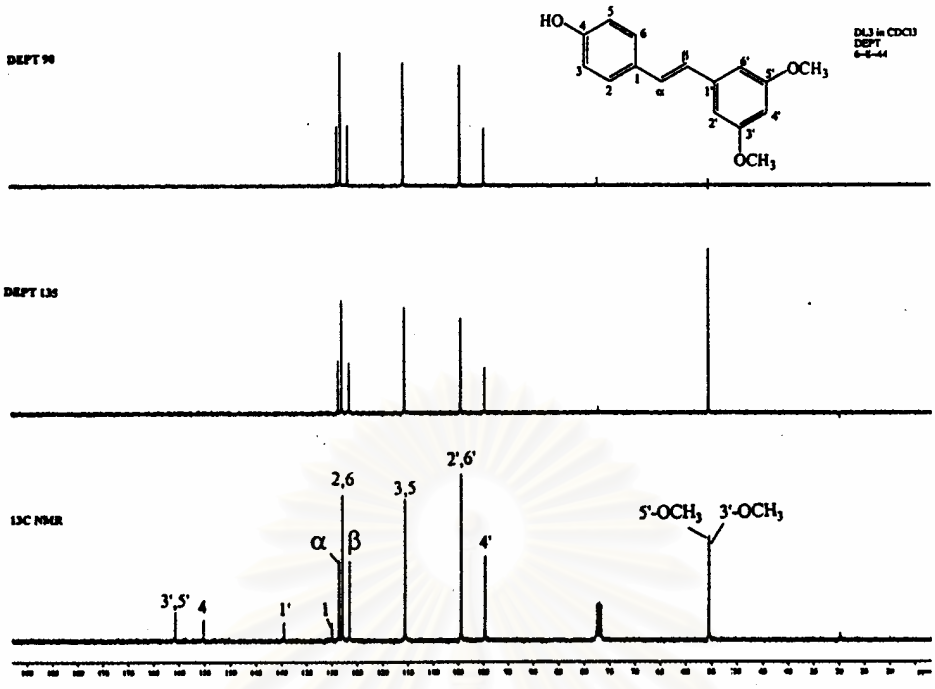

Figure 15 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-B (in $CDCl_3$)

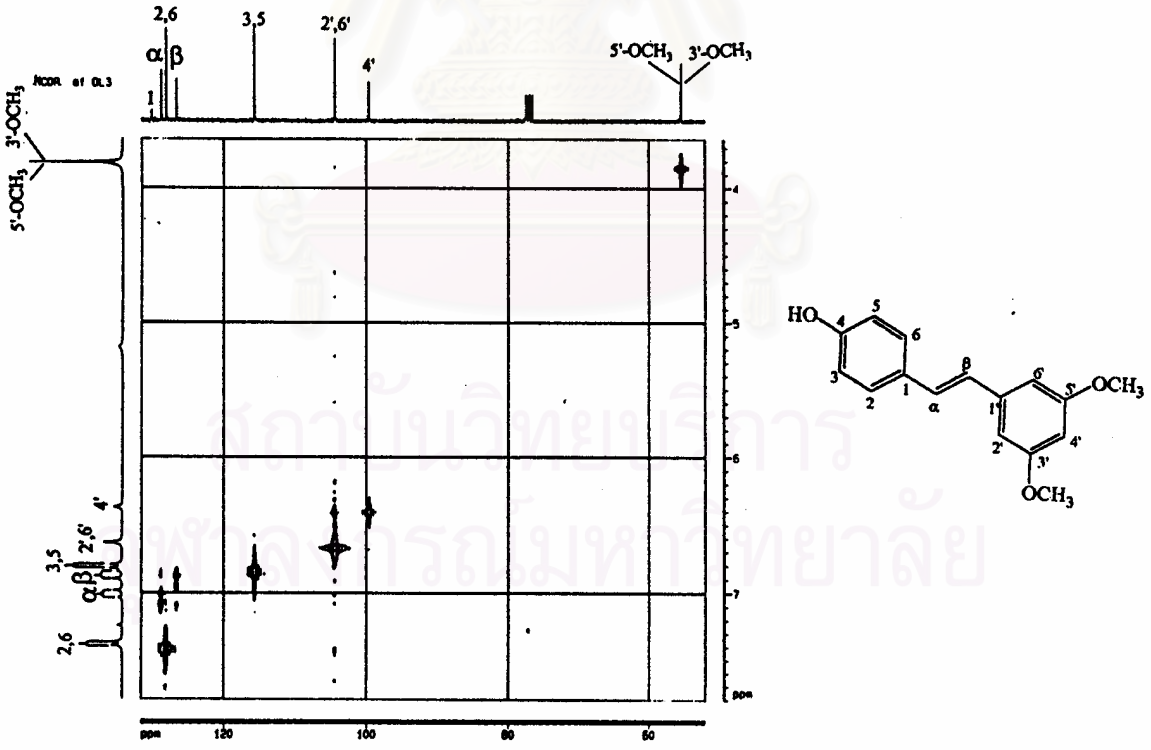

Figure 16a HETCOR spectrum of compound DL-B (in $CDCl_3$)

[δ_H 3.7-7.7 ppm, δ_C 50-130 ppm]

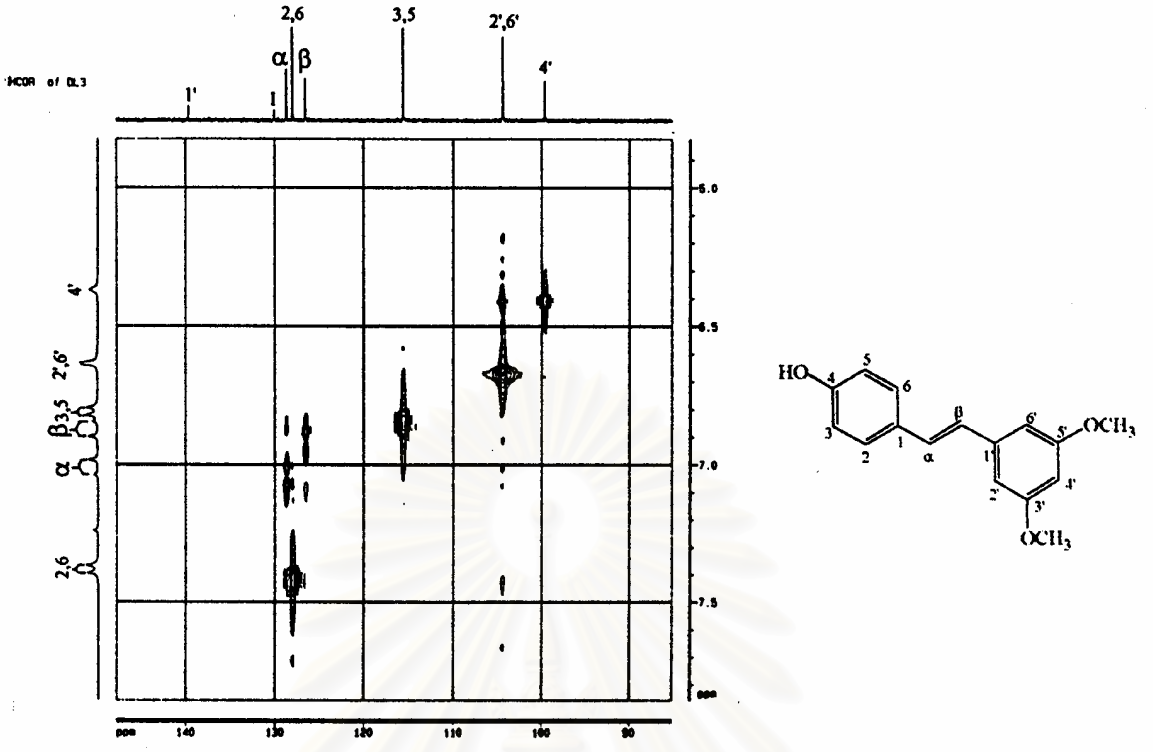

Figure 16b HETCOR spectrum of compound DL-B (in $CDCl_3$)

[δ_H 5.8-7.7 ppm, δ_C 85-148 ppm]

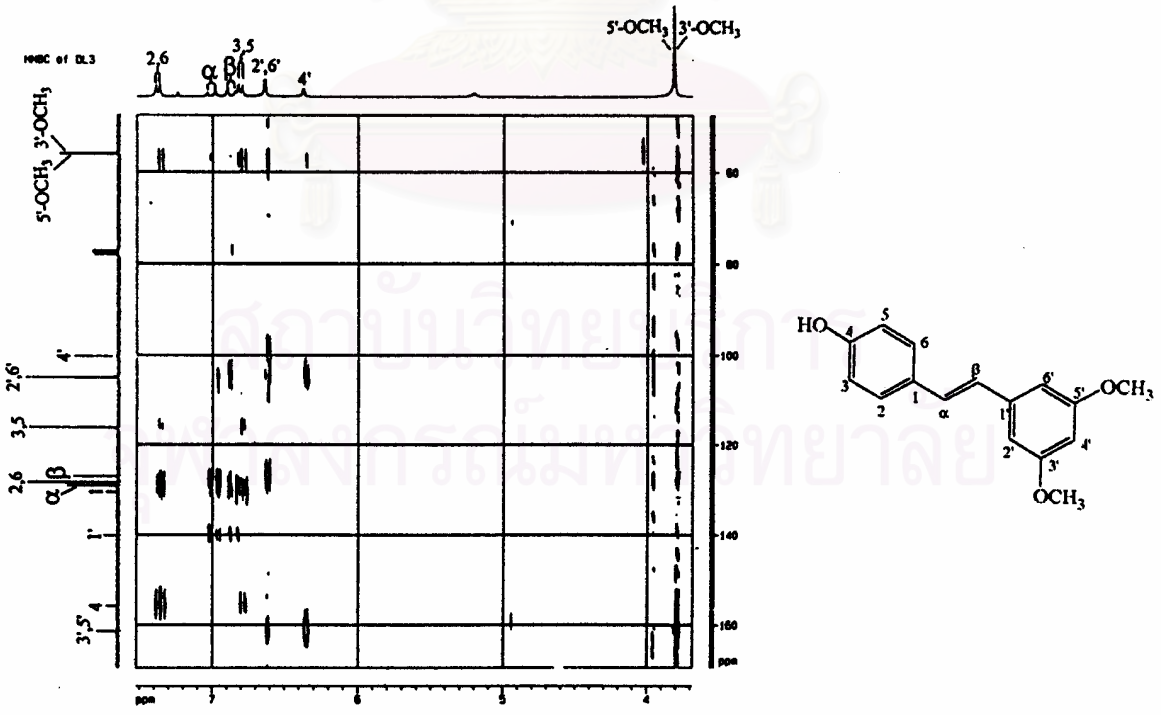

Figure 17 HMBC spectrum of compound DL-B (in $CDCl_3$)

[δ_H 3.7-7.7 ppm, δ_C 50-170 ppm]

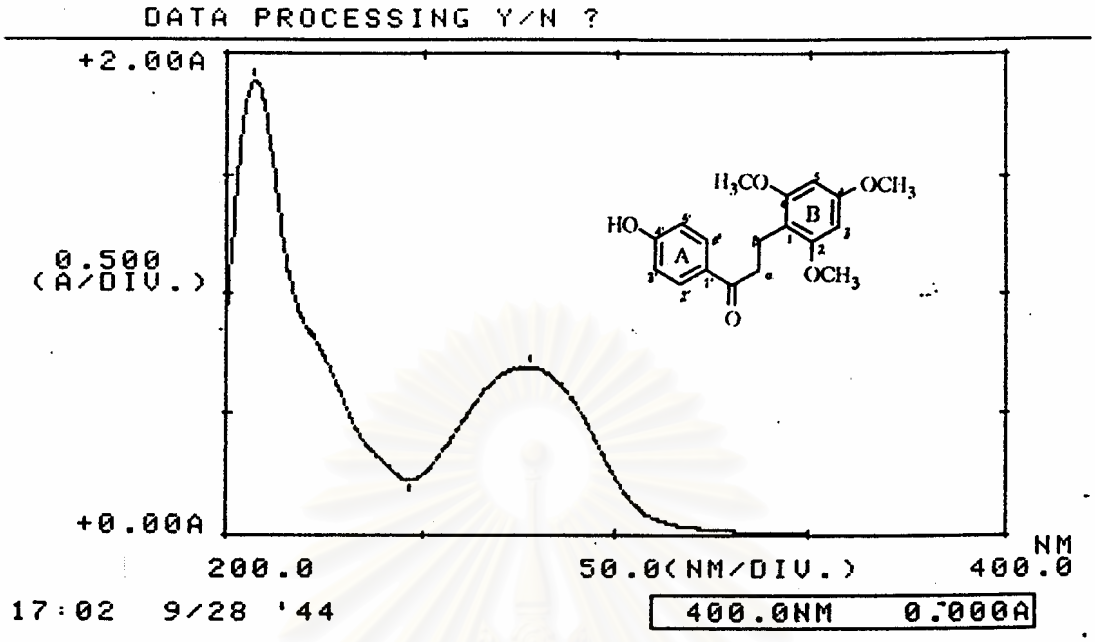

Figure 18 UV spectrum of compound DL-C (in methanol)

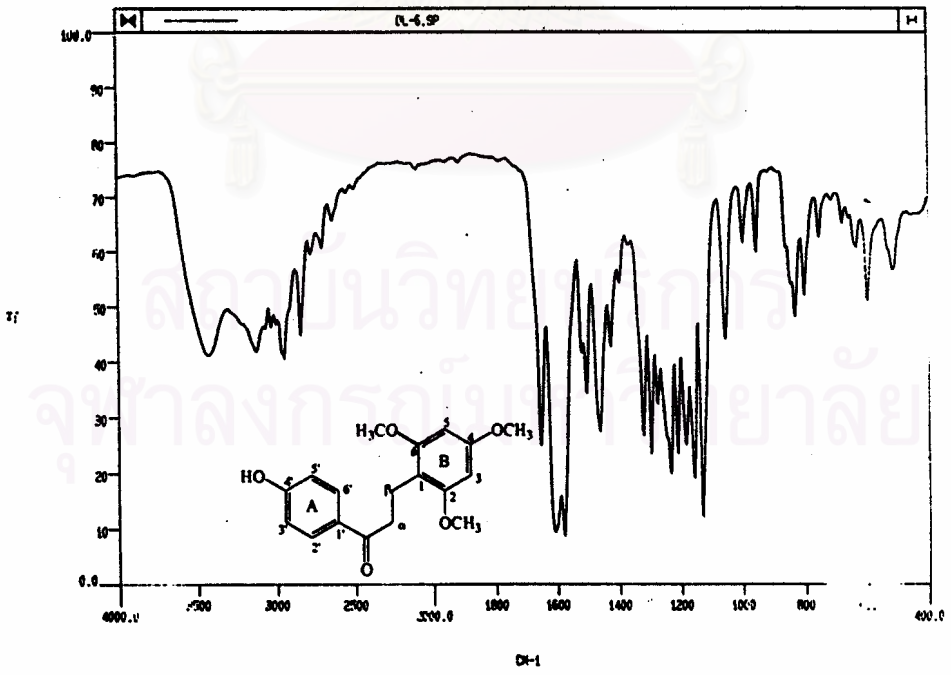

Figure 19 IR spectrum of compound DL-C (KBr disc)

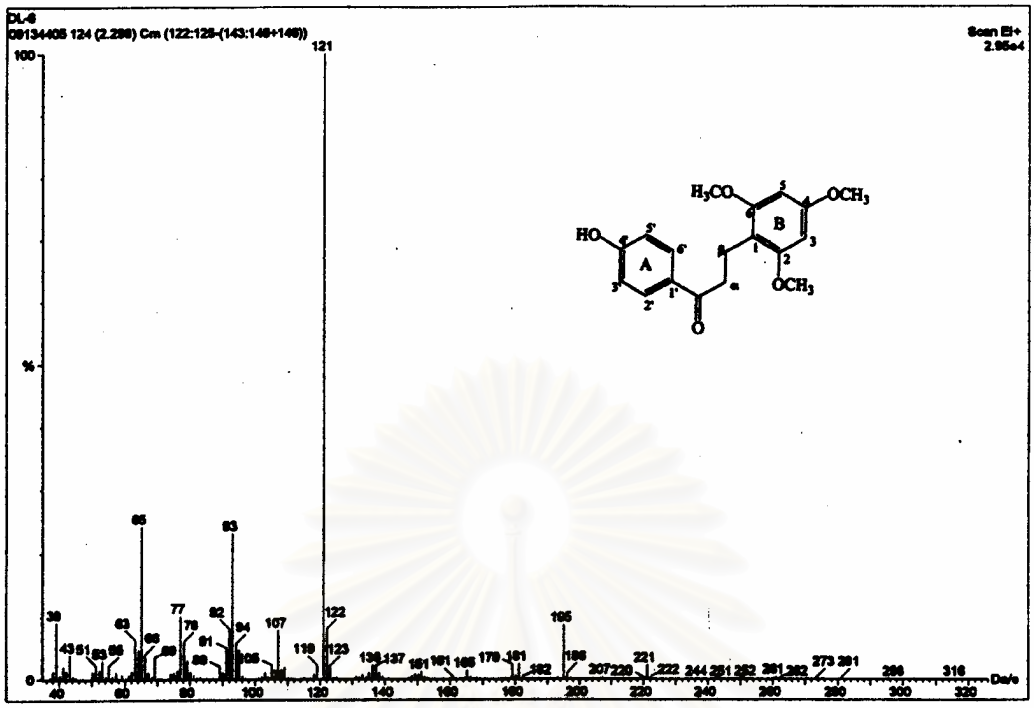

Figure 20 EI mass spectrum of compound DL-C

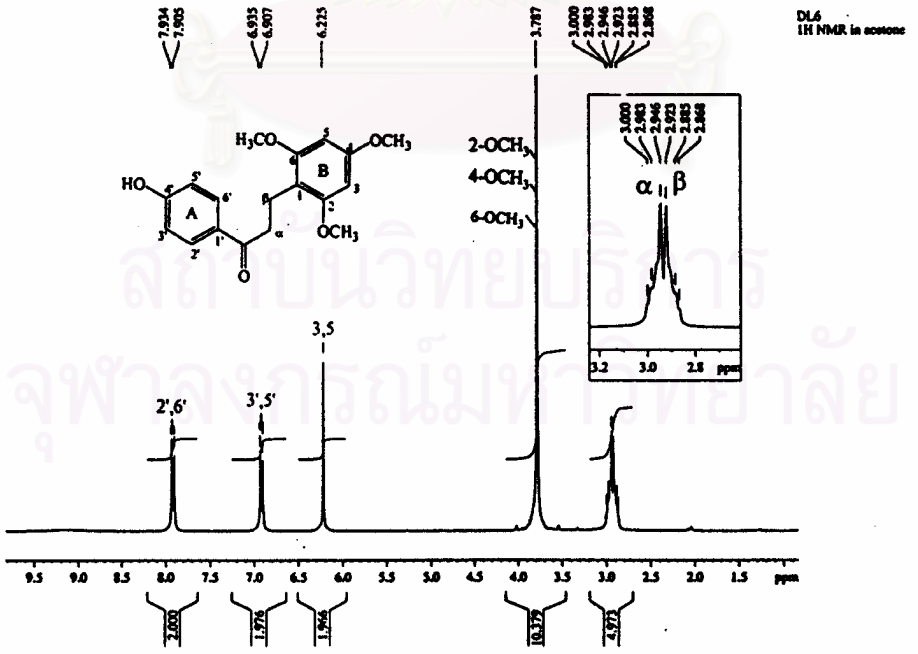

Figure 21 300 MHz ^{1}H NMR spectrum of compound DL-C (in acetone-d_6)

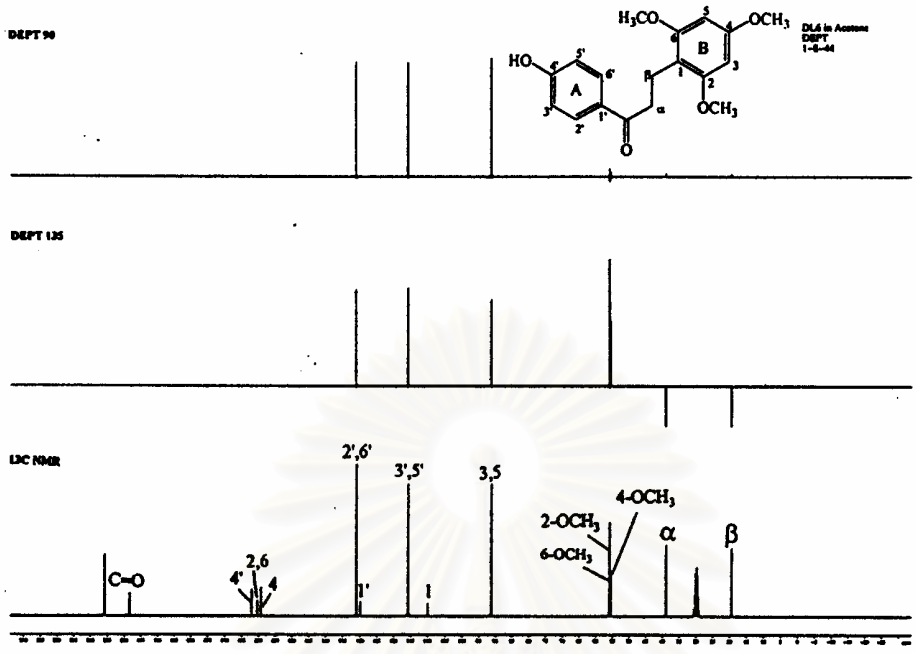

Figure 22 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-C (in acetone-d_6)

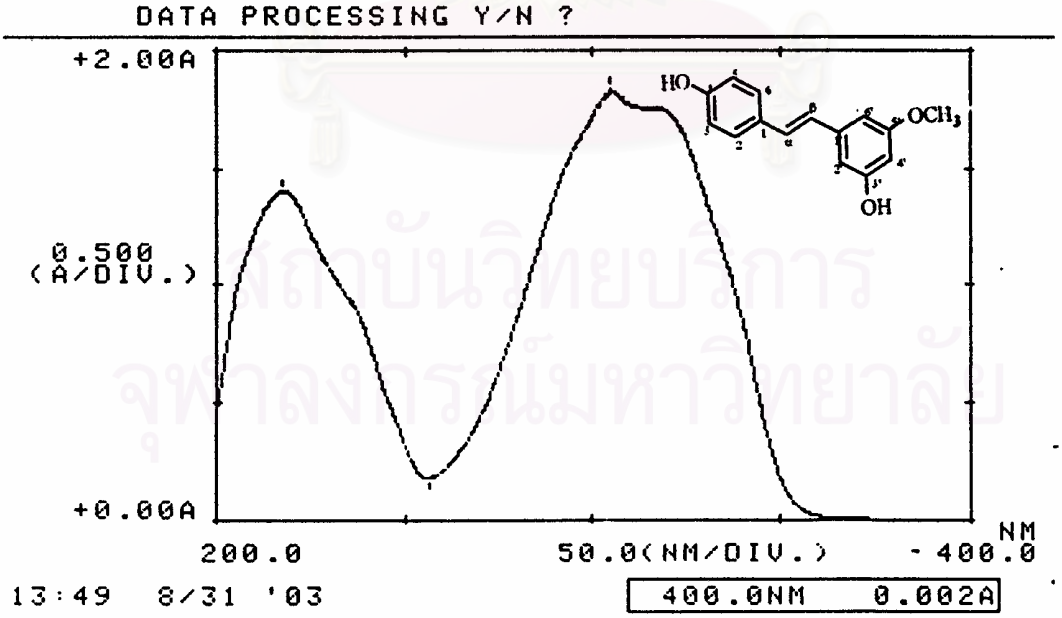

Figure 23 UV spectrum of compound DL-D (in methanol)

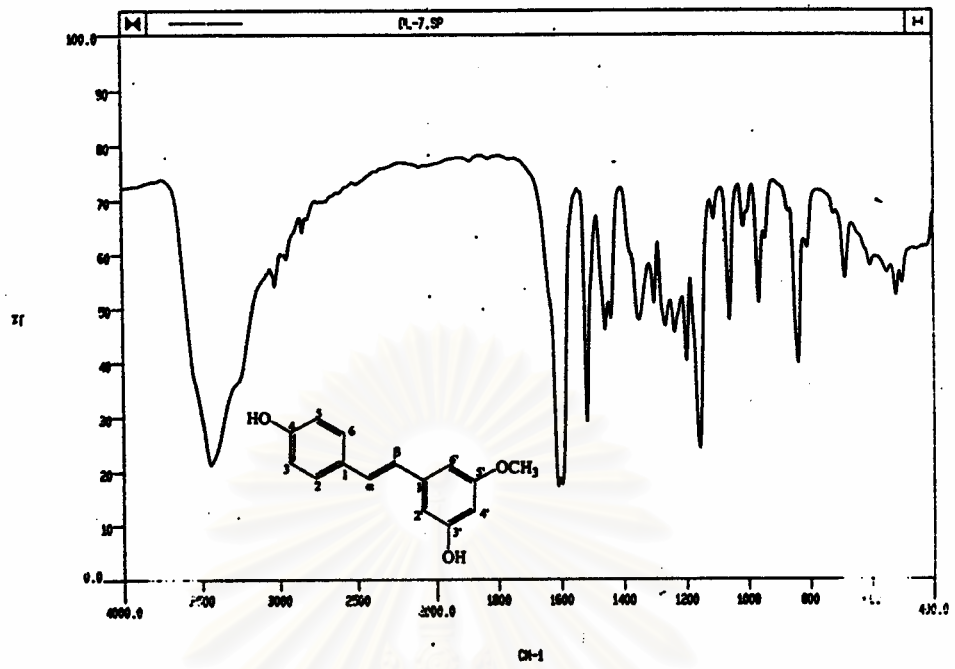

Figure 24 IR spectrum of compound DL-D (KBr disc)

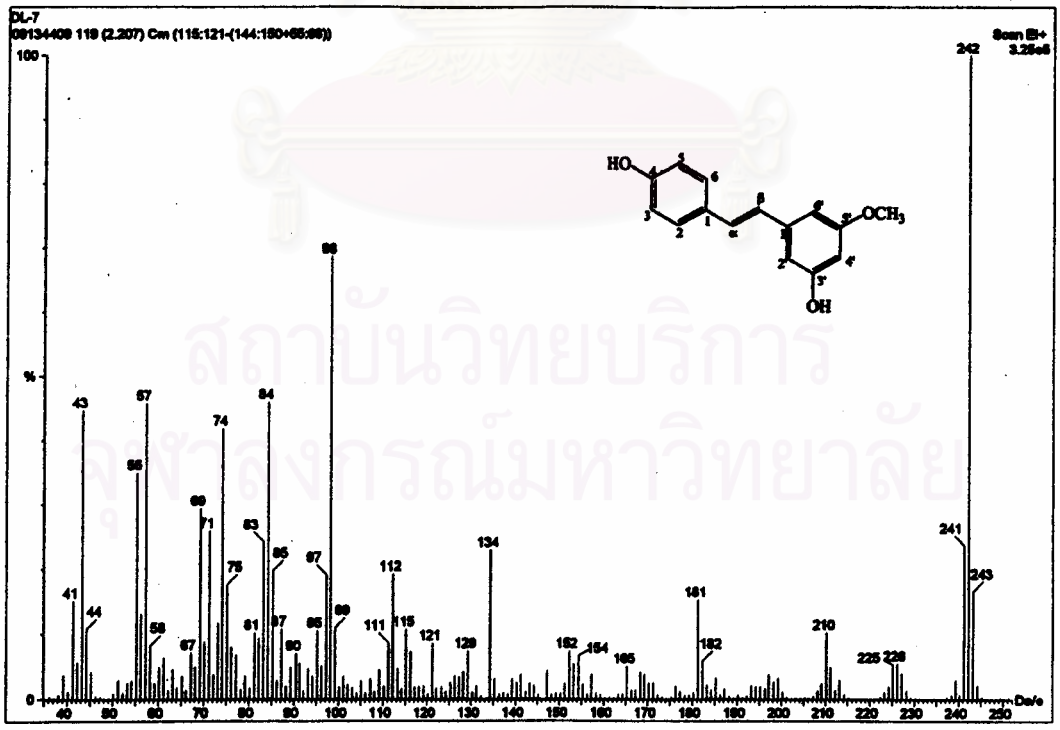

Figure 25 EI mass spectrum of compound DL-D

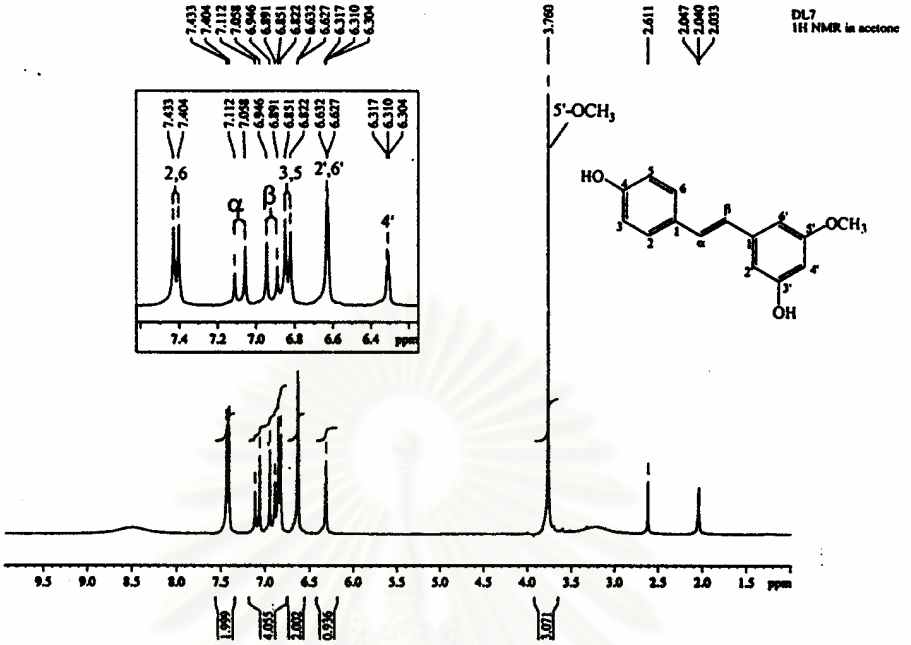

Figure 26 300 MHz ^{1}H NMR spectrum of compound DL-D (in acetone-d_6)

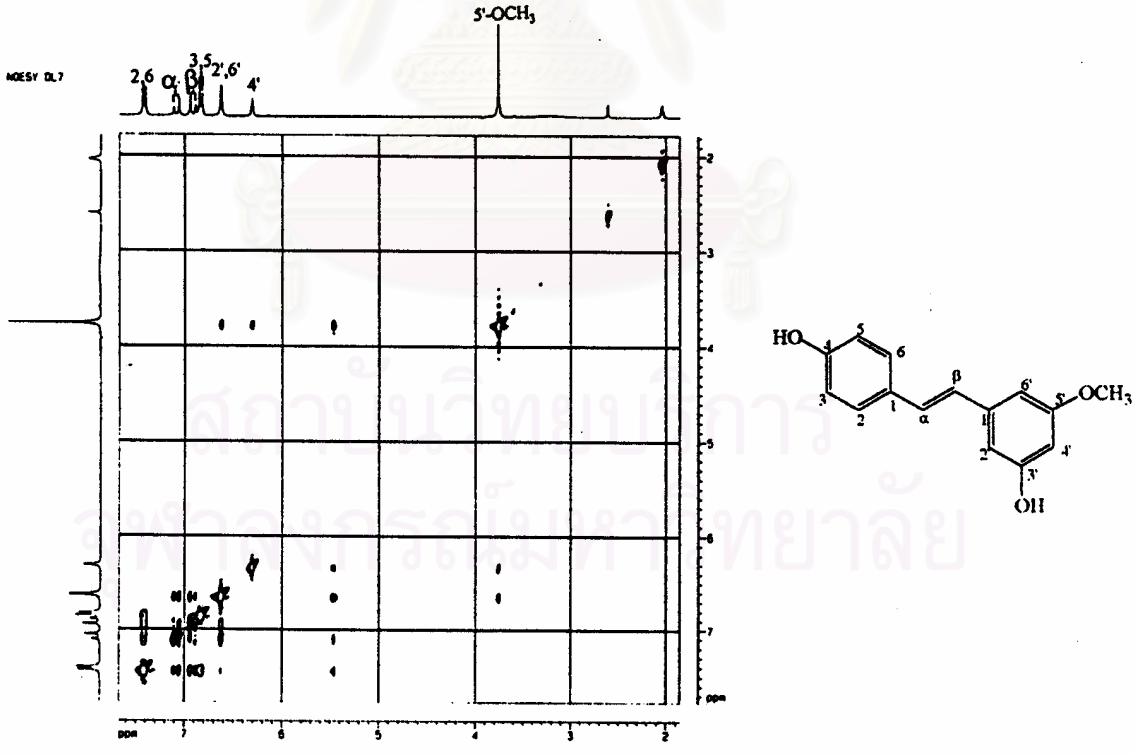

Figure 27a NOESY spectrum of compound DL-D (in acetone-d_6)

(expanded from δ_H 2.0-7.6 ppm)

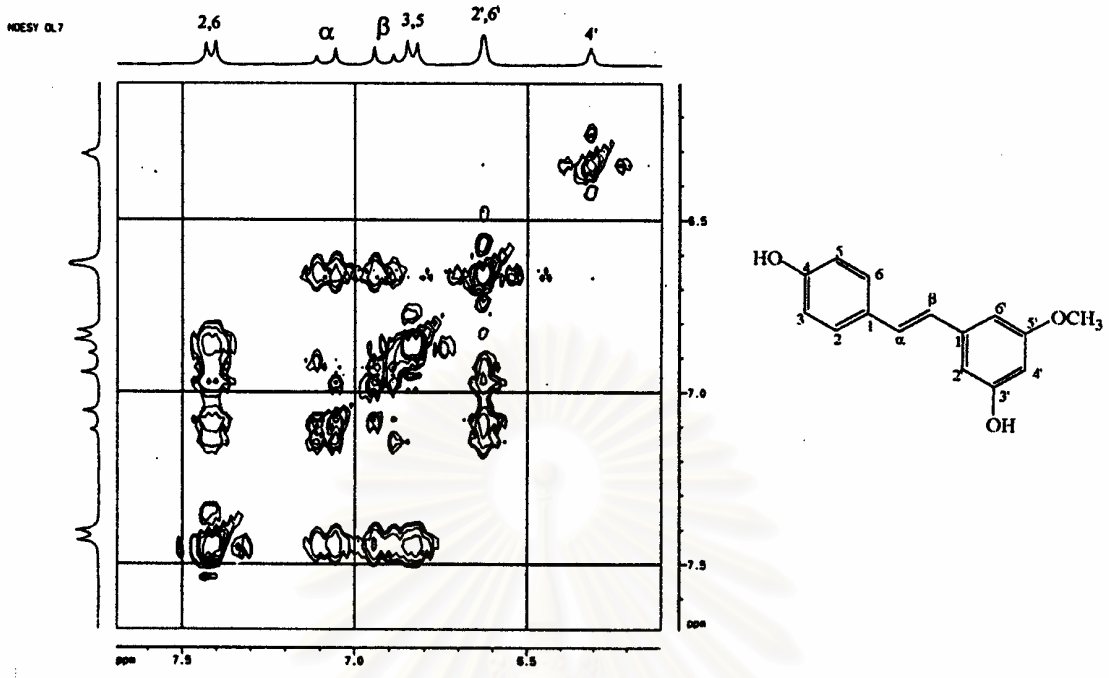

Figure 27b NOESY spectrum of compound DL-D (in acetone-d_6)

(expanded from δ_H 6.1-7.7 ppm)

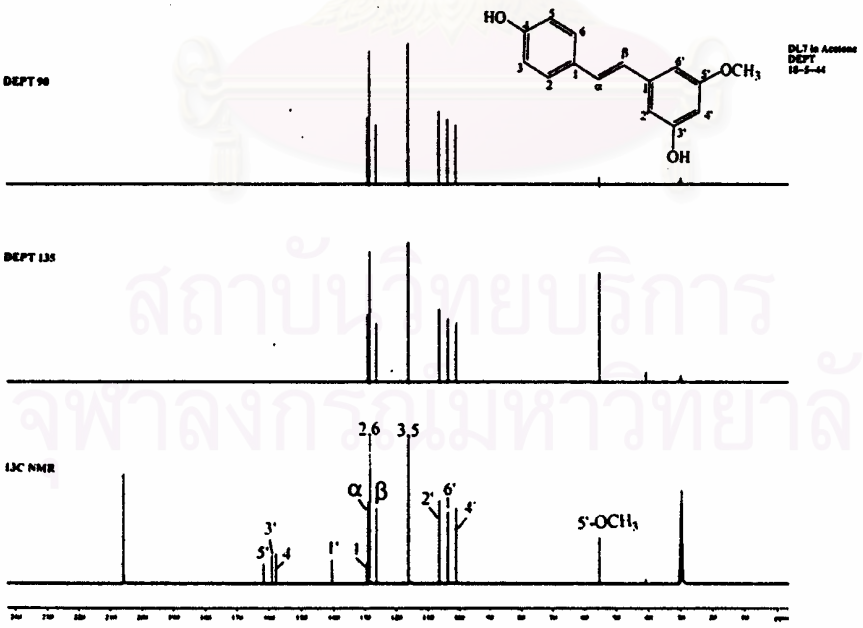

Figure 28 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-D (in acetone-d_6)

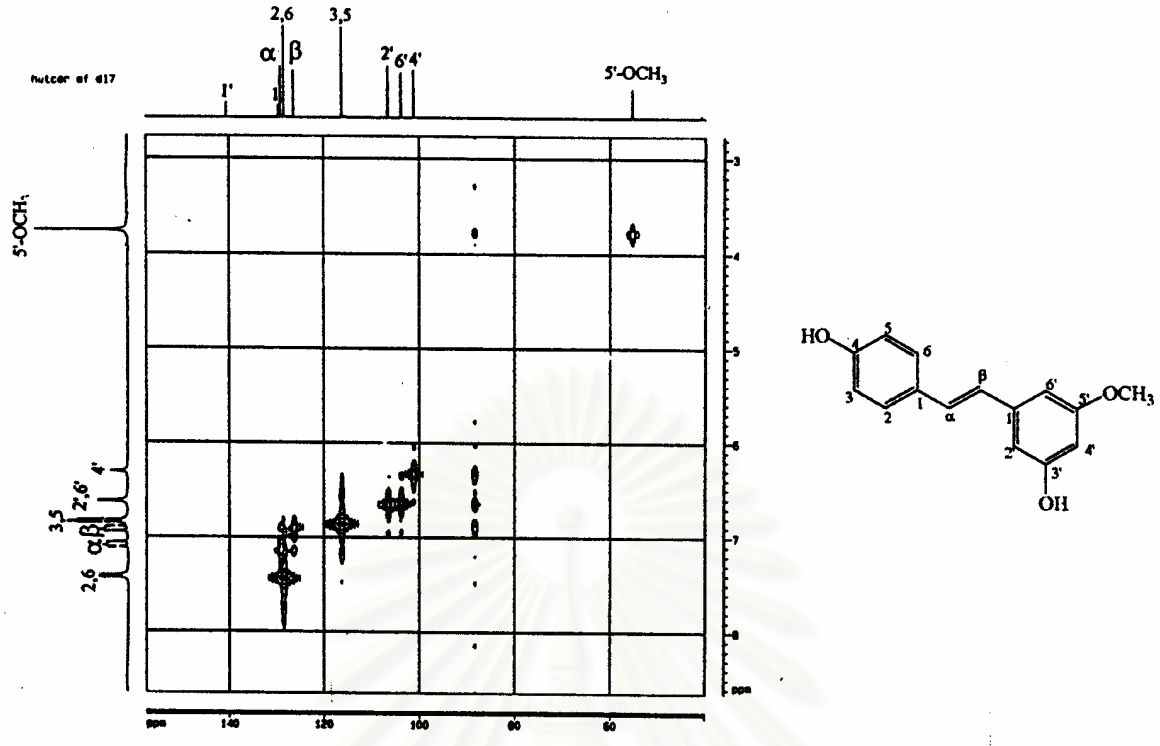

Figure 29a HETCOR spectrum of compound DL-D (in acetone-d_6)

[δ_H 2.8-8.5 ppm, δ_C 40-160 ppm]

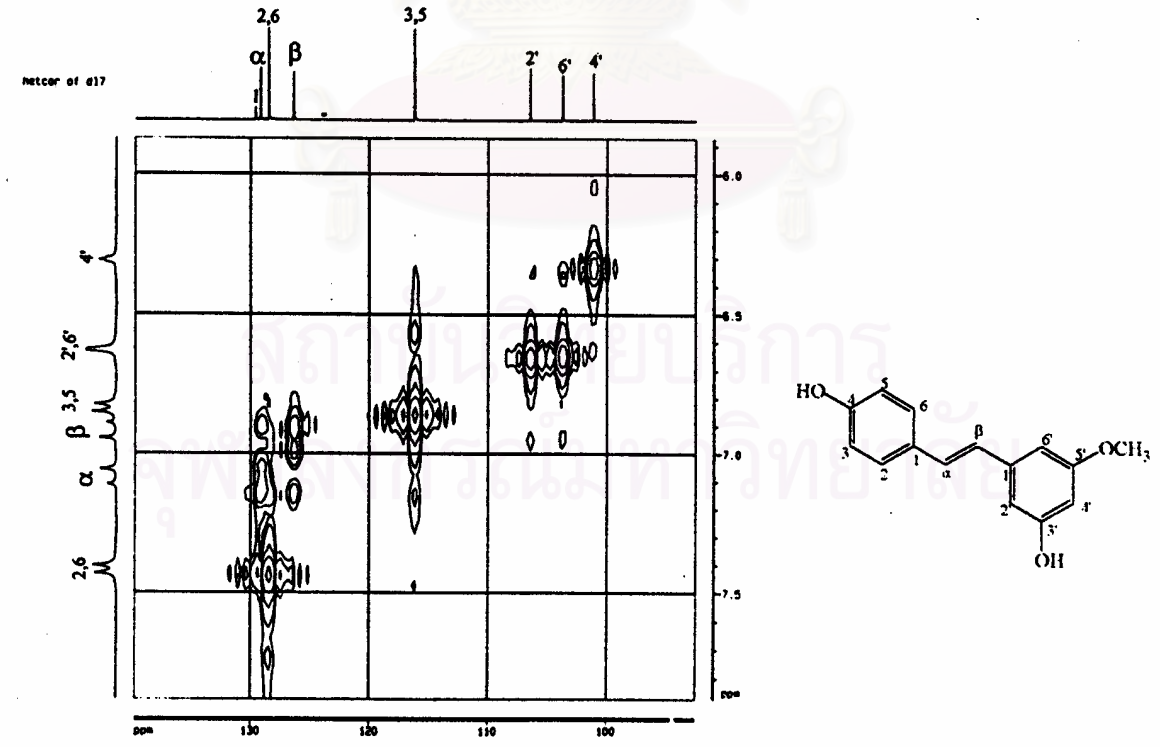

Figure 29b HETCOR spectrum of compound DL-D (in acetone-d_6)

[δ_H 5.9-7.8 ppm, δ_C 90-140 ppm]

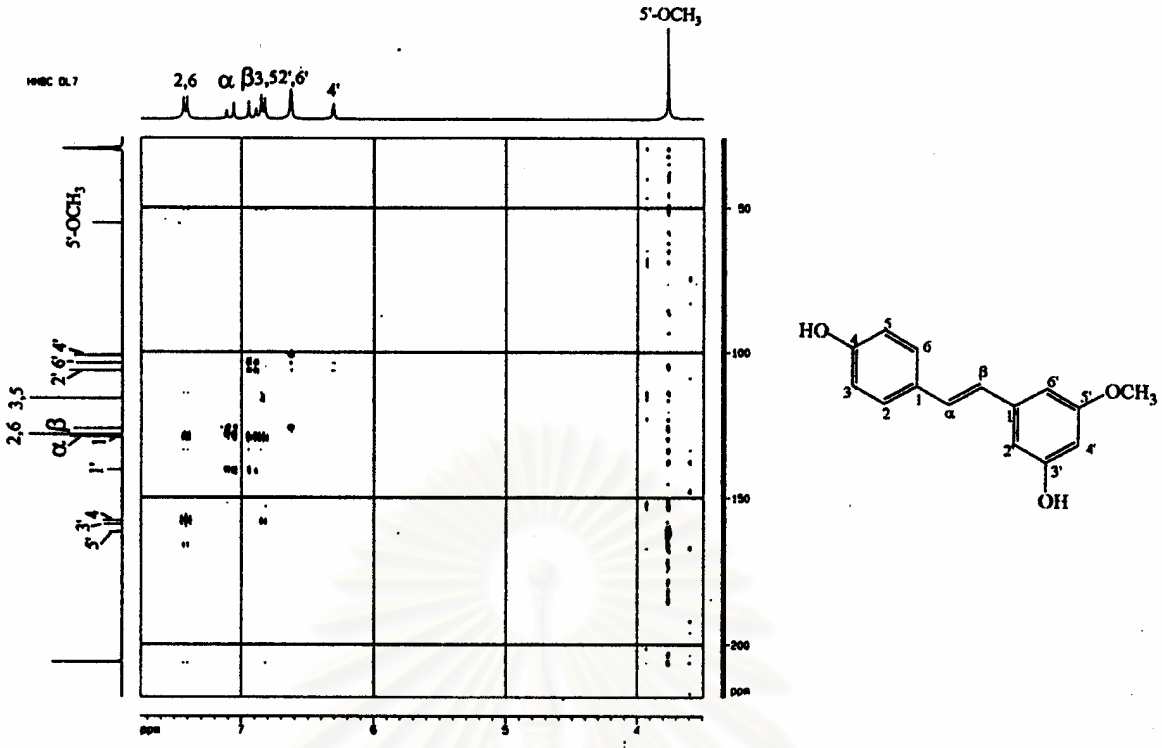

Figure 30a HMBC spectrum of compound DL-D (in acetone-d_6)

[δ_H 3.5-7.7 ppm, δ_C 27-220 ppm]

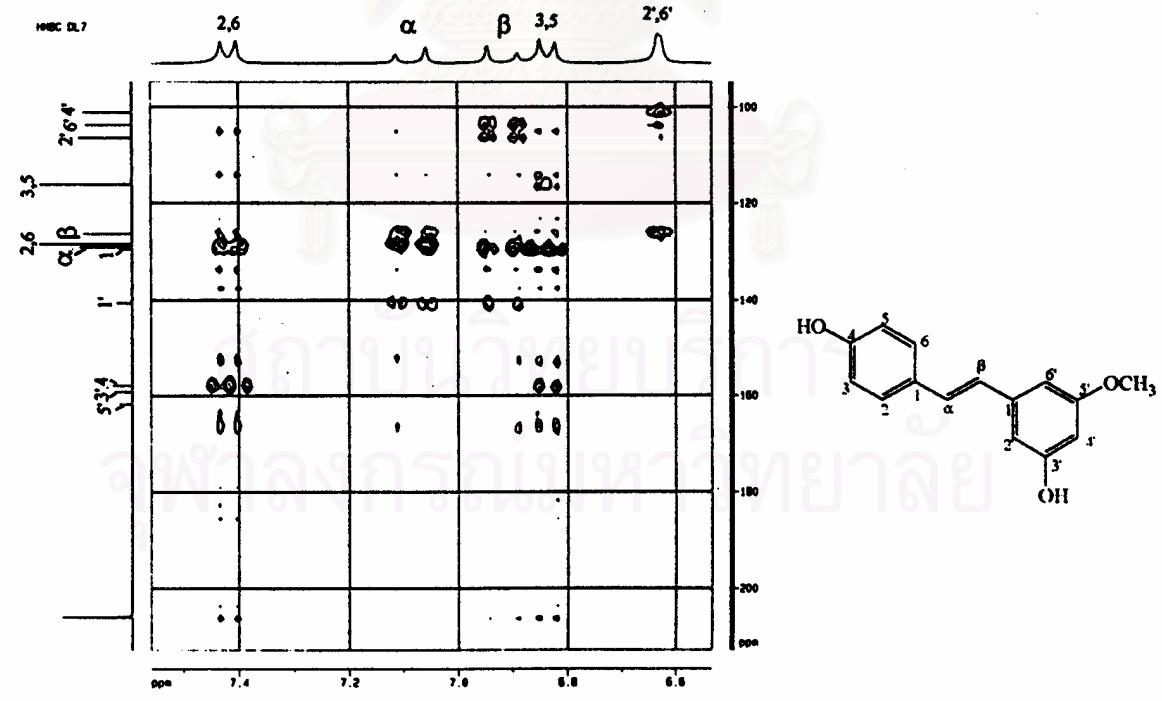

Figure 30b HMBC spectrum of compound DL-D (in acetone-d_6)

[δ_H 6.5-7.5 ppm, δ_C 95-220 ppm]

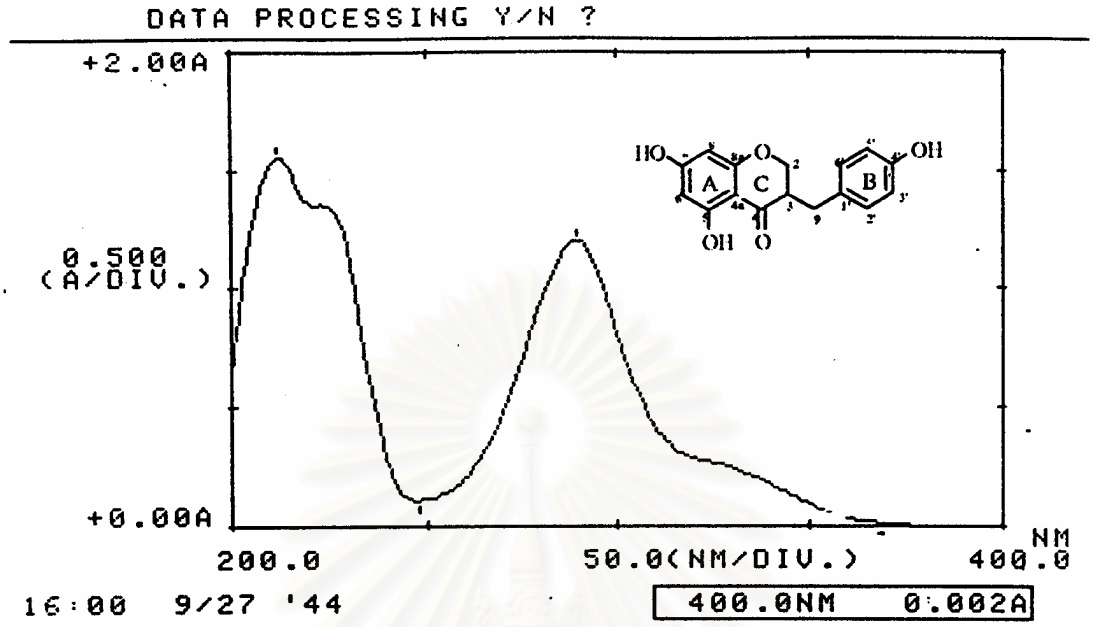

Figure 31 UV spectrum of compound DL-E (in methanol)

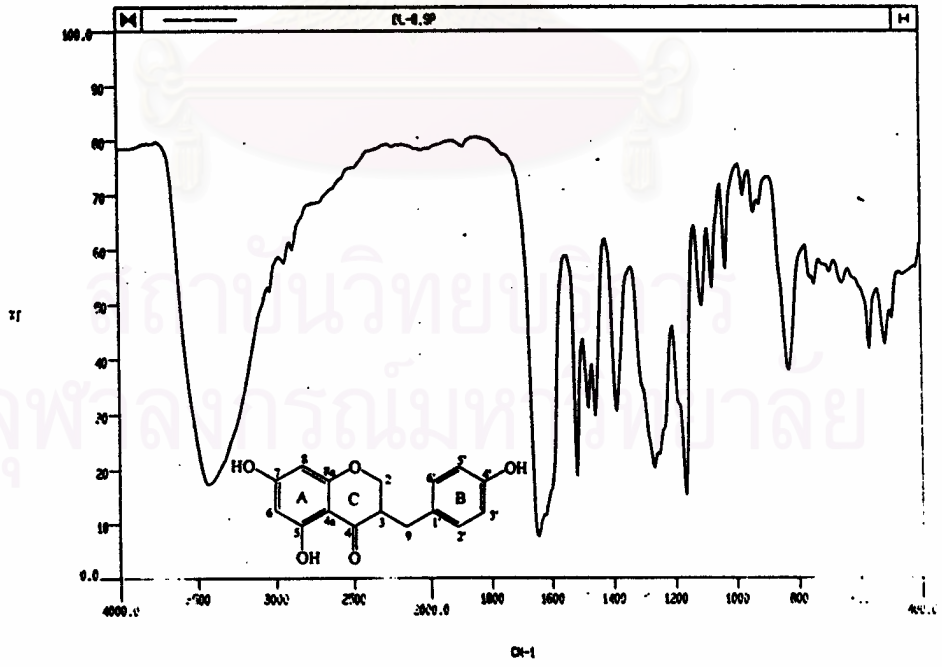

Figure 32 IR spectrum of compound DL-E (KBr disc)

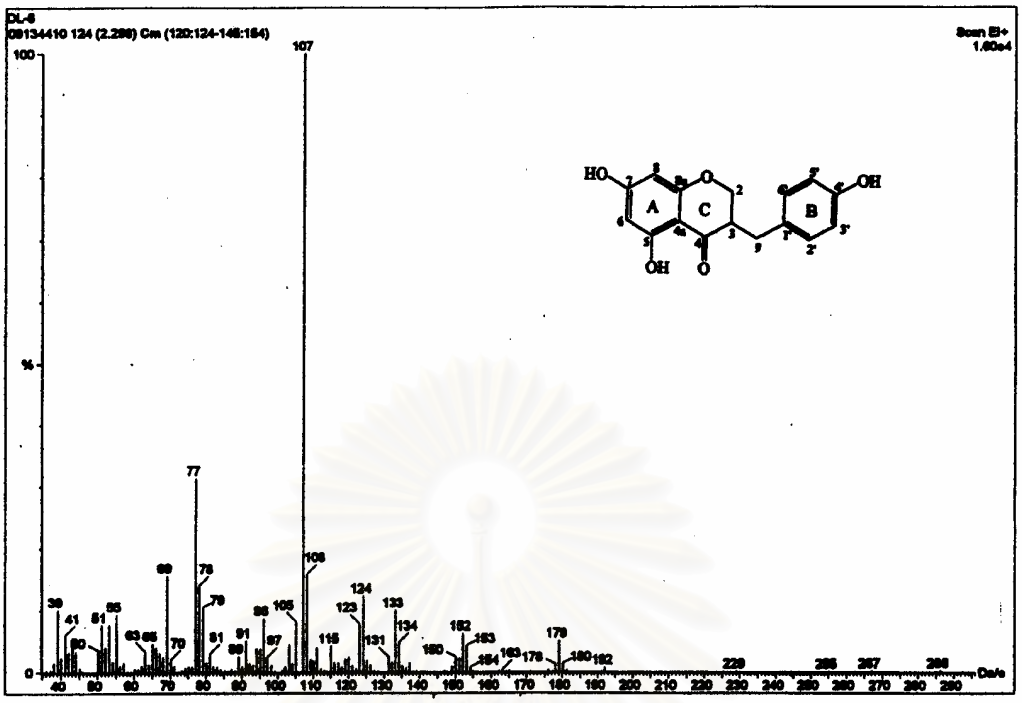

Figure 33 EI mass spectrum of compound DL-E

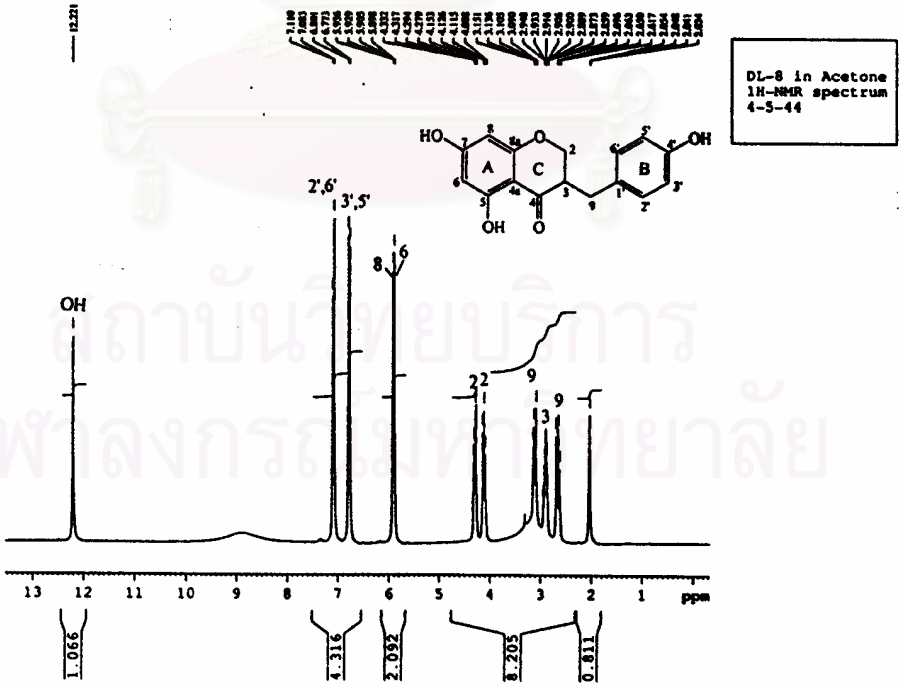

Figure 34a 300 MHz ^{1}H NMR spectrum of compound DL-E (in acetone-d_6)

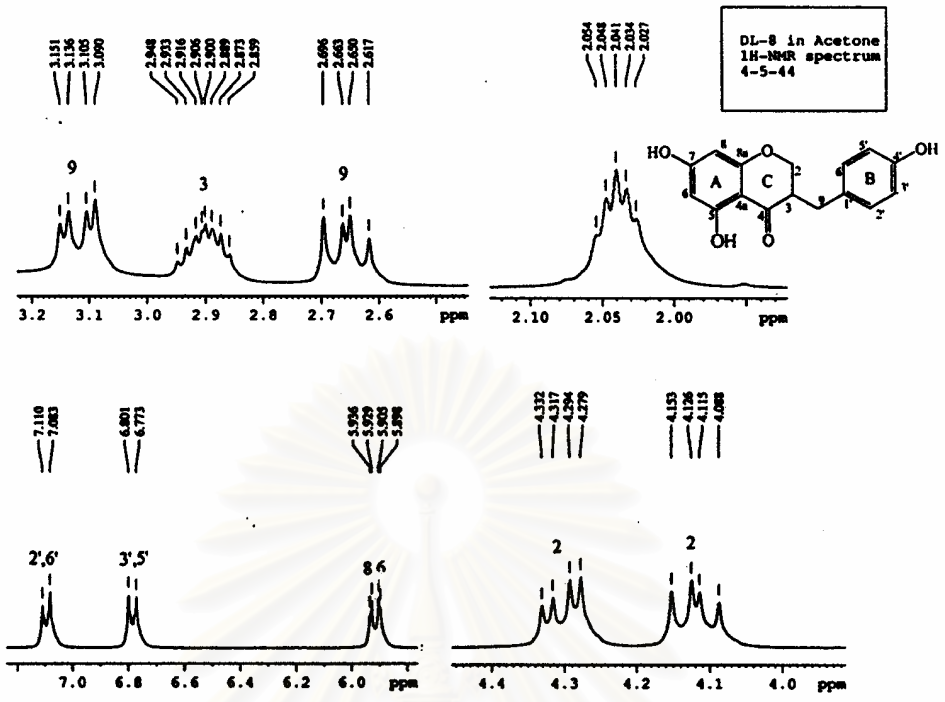

Figure 34b 300 MHz ^{1}H NMR spectrum of compound DL-E (in acetone-d_6)

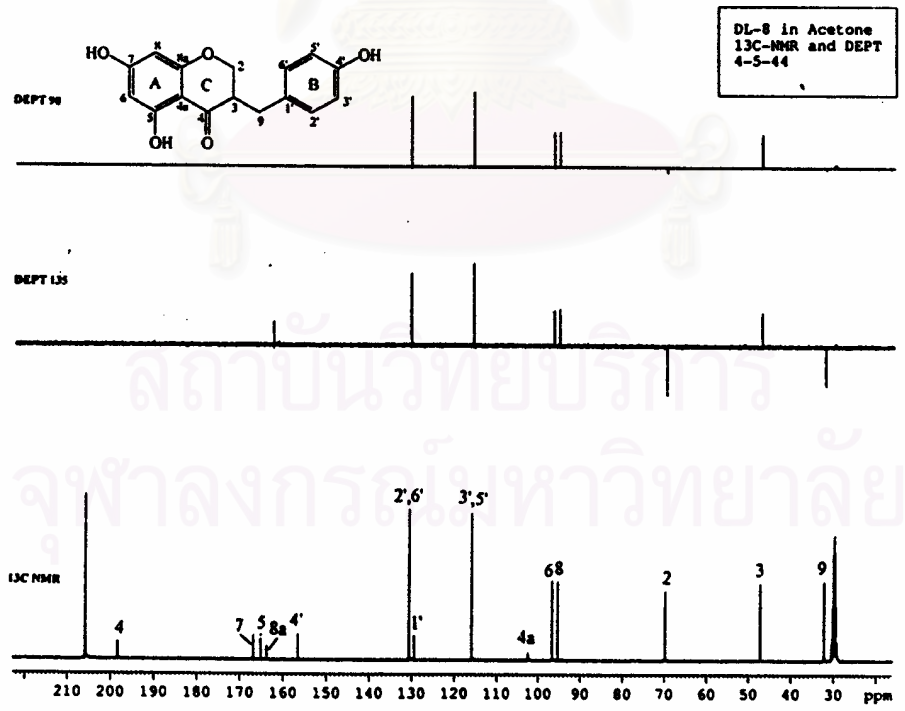

Figure 35 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-E (in acetone-d_6)

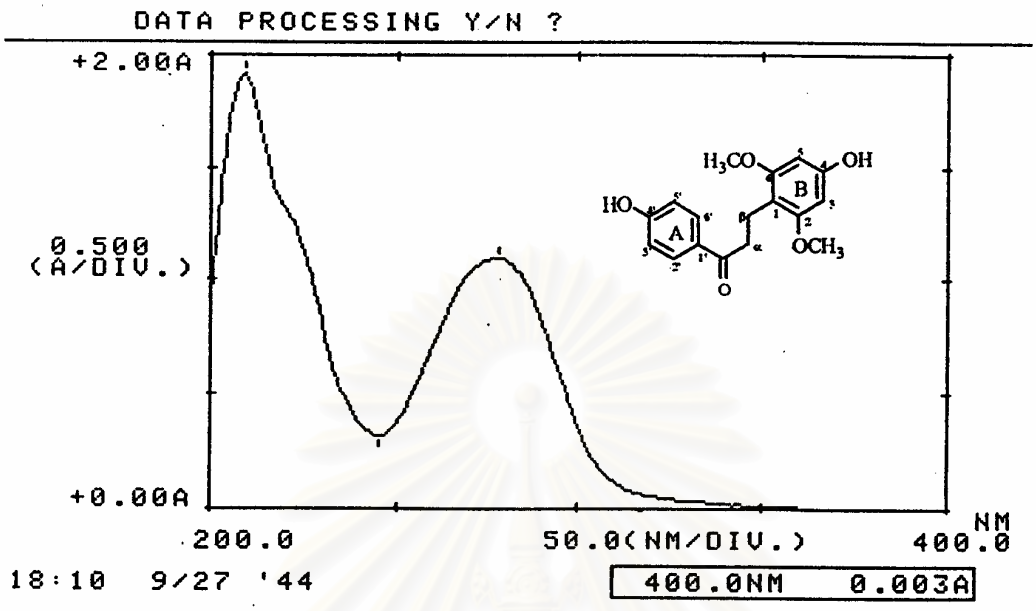

Figure 36 .UV spectrum of compound DL-F (in methanol)

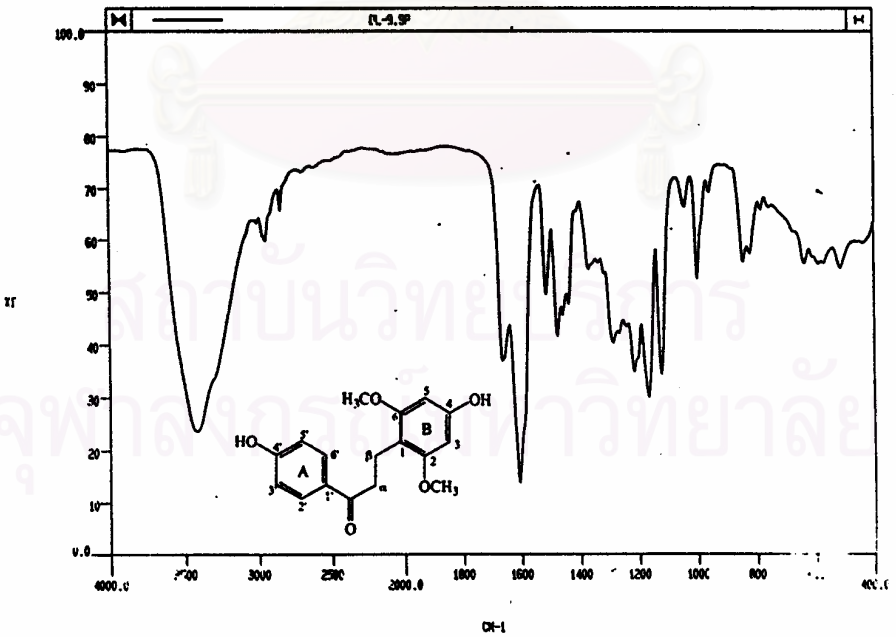

Figure 37 IR spectrum of compound DL-F (KBr disc)

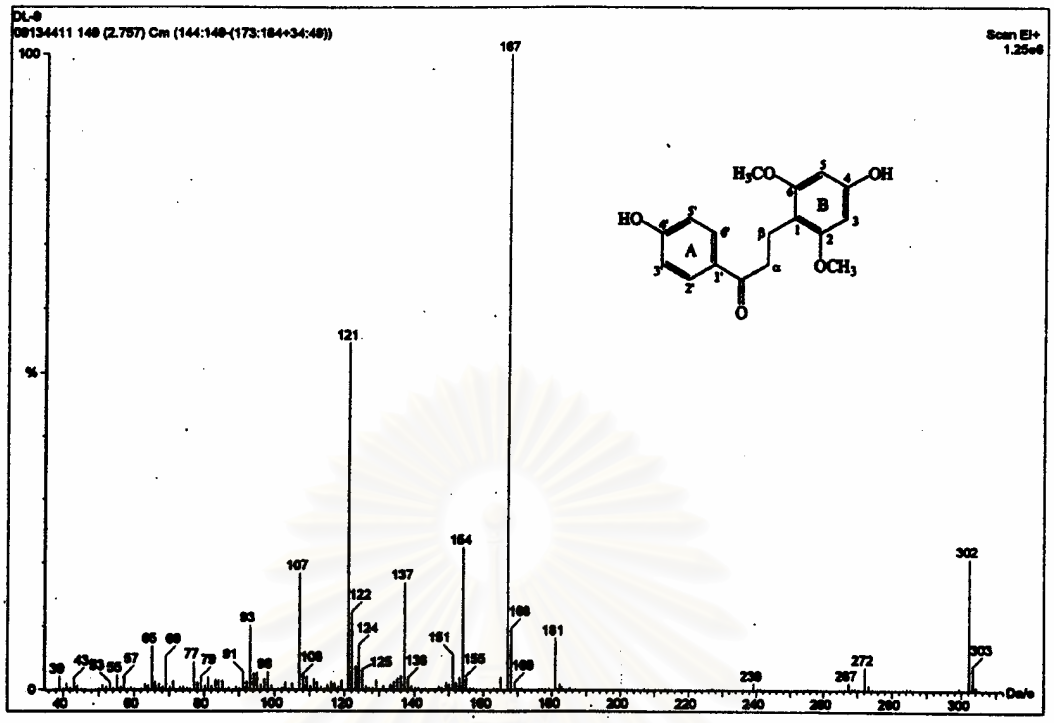

Figure 38 EI mass spectrum of compound DL-F

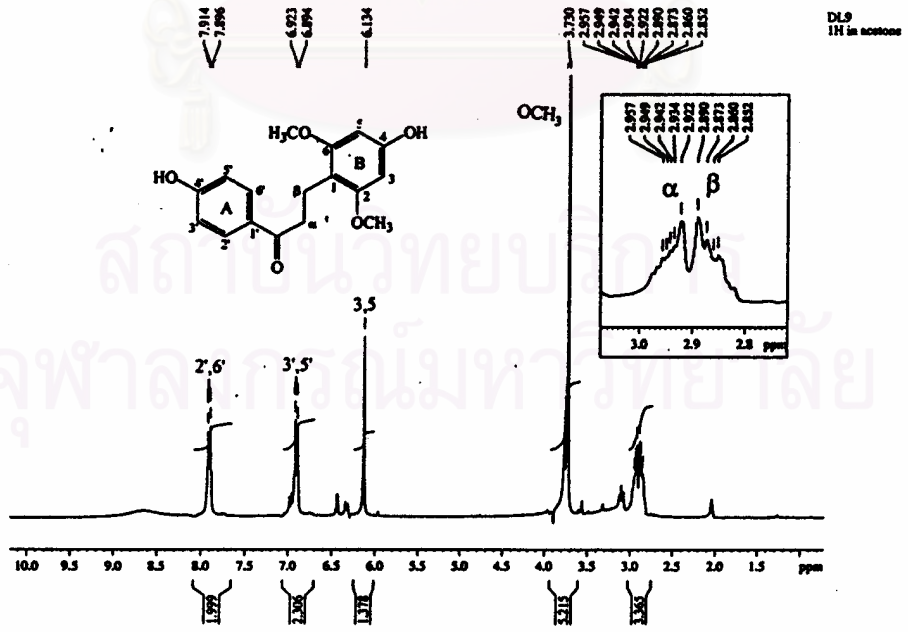

Figure 39 300 MHz ^{1}H NMR spectrum of compound DL-F (in acetone-d_6)

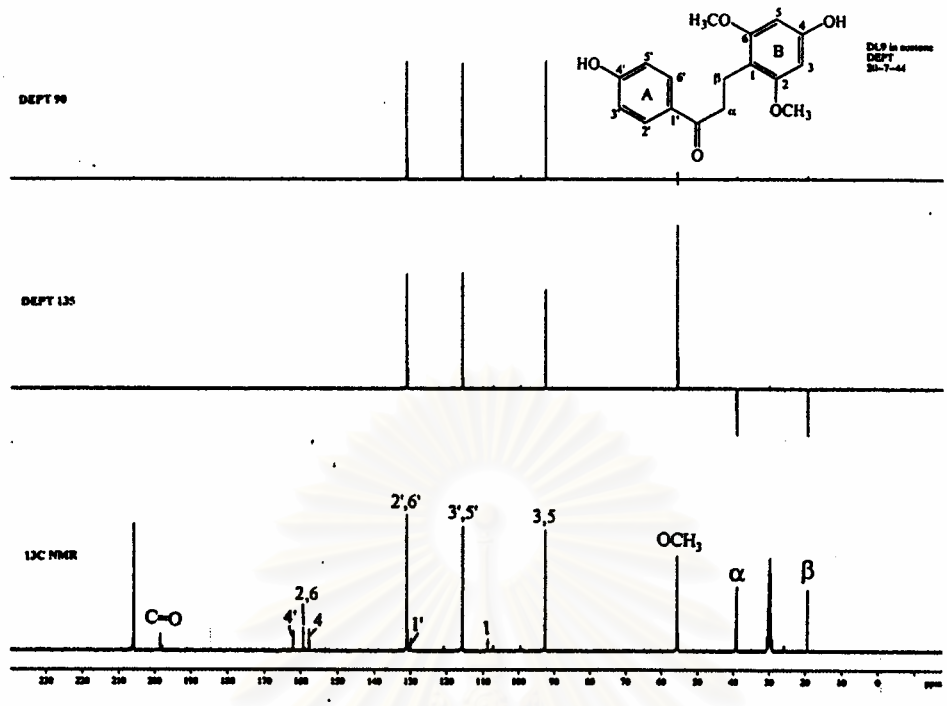

Figure 40 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-F (in acetone-d_6)

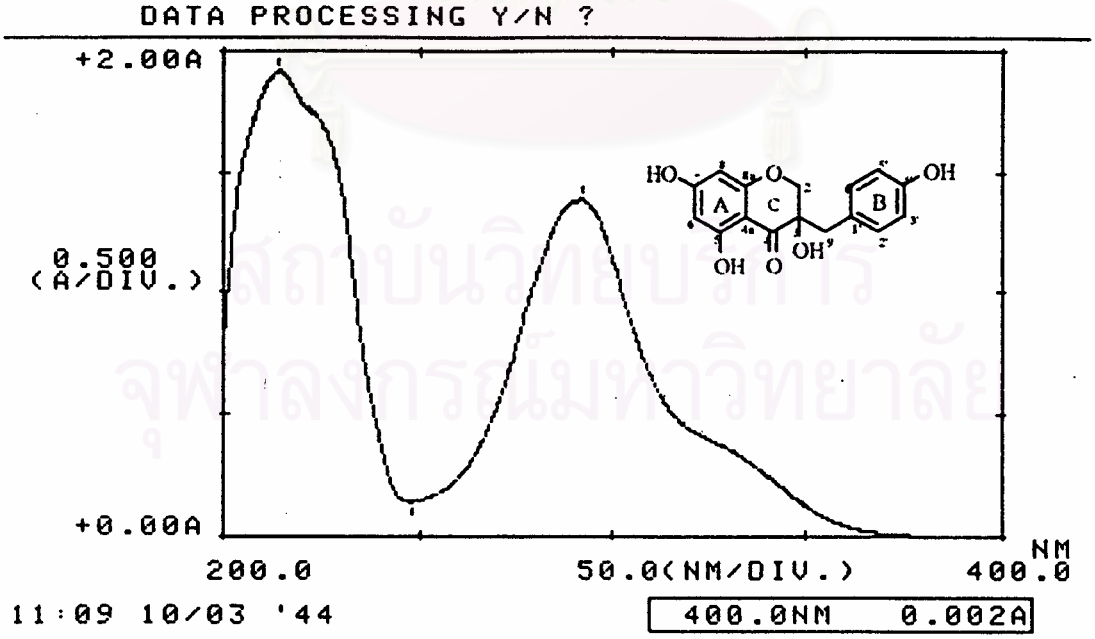

Figure 41 UV spectrum of compound DL-G (in methanol)

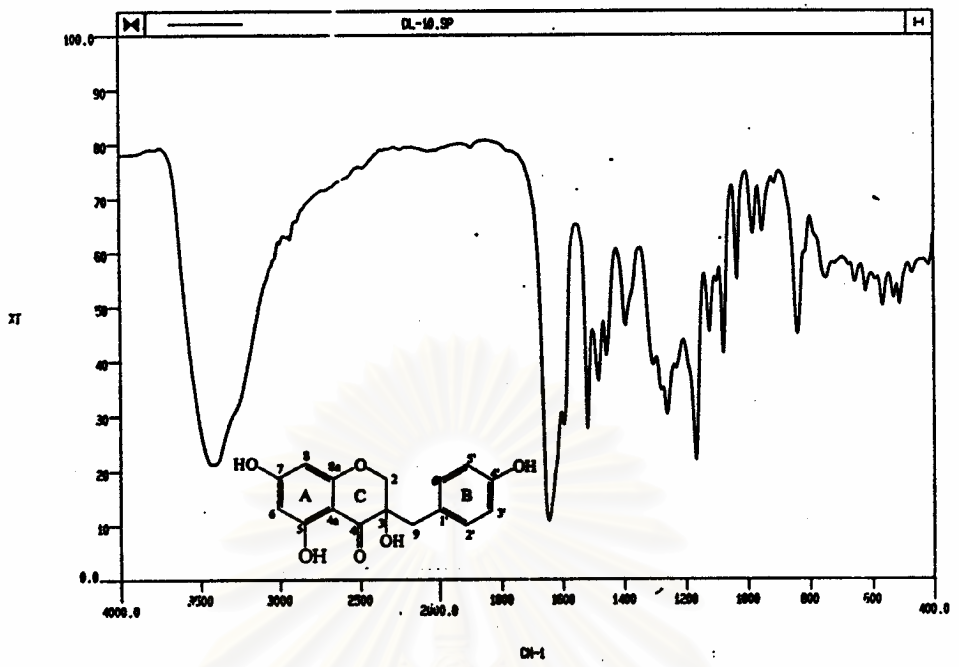

Figure 42 IR spectrum of compound DL-G (KBr disc)

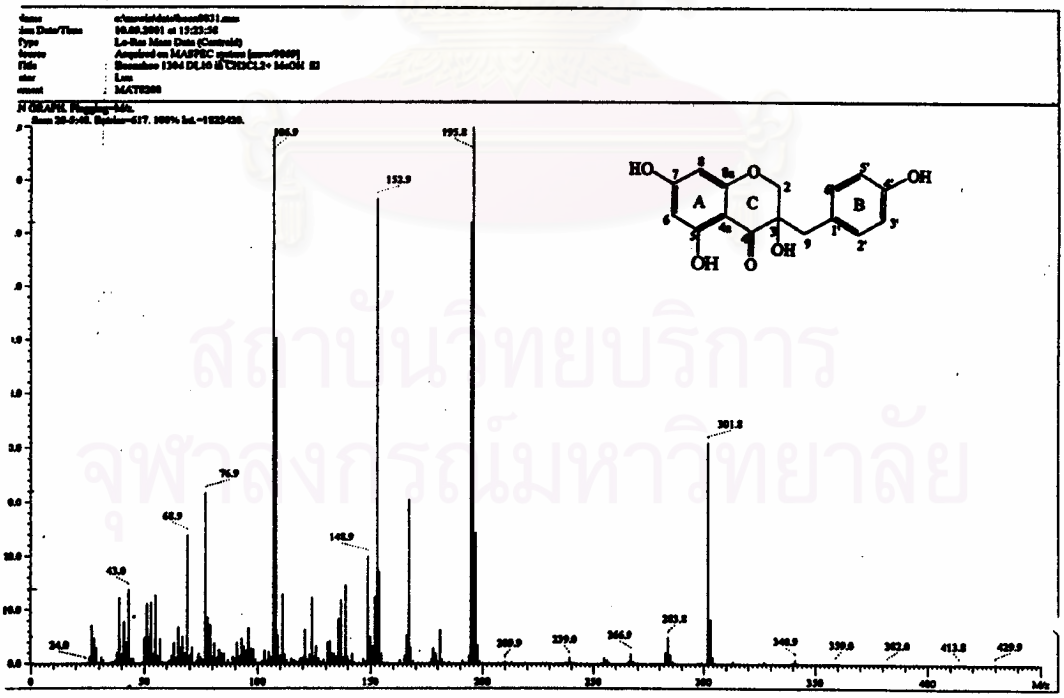

Figure 43 HREIMS spectrum of compound DL-G

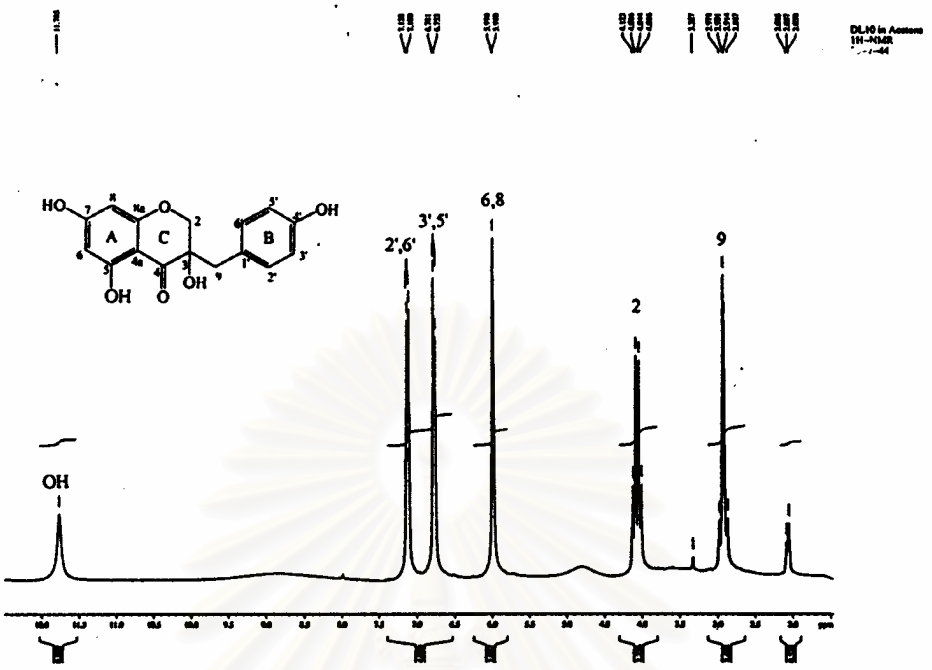

Figure 44 300 MHz ^{1}H NMR spectrum of compound DL-G (in acetone-d_6)

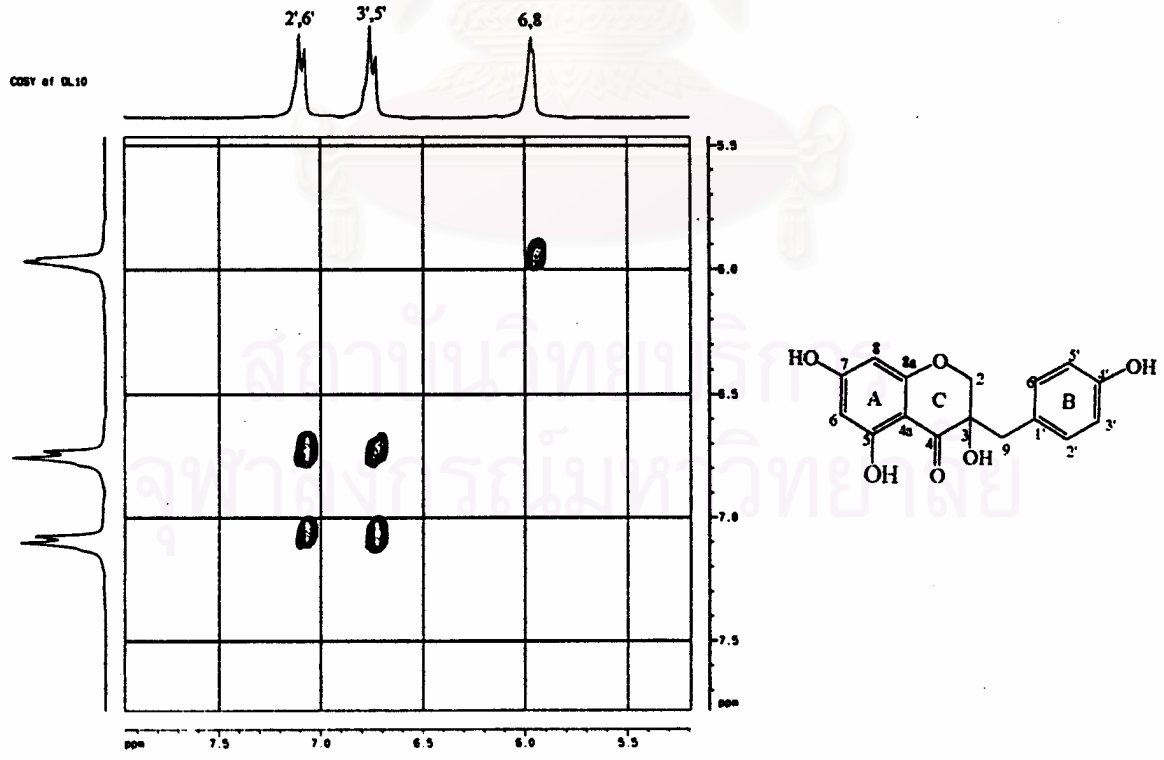

Figure 45 ^{1}H-^{1}H COSY spectrum of compound DL-G (in acetone-d_6)

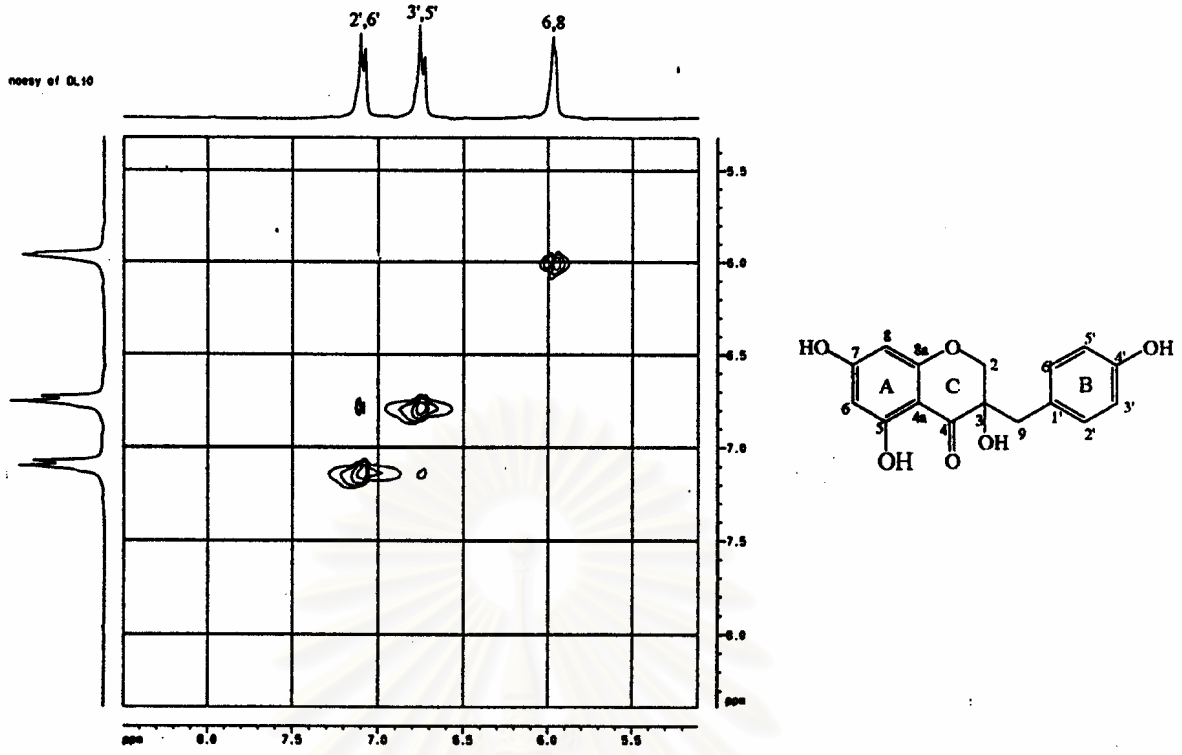

Figure 46 NOESY spectrum of compound DL-G (in acetone-d_6)
(expanded from δ_H 5.4-8.3 ppm)

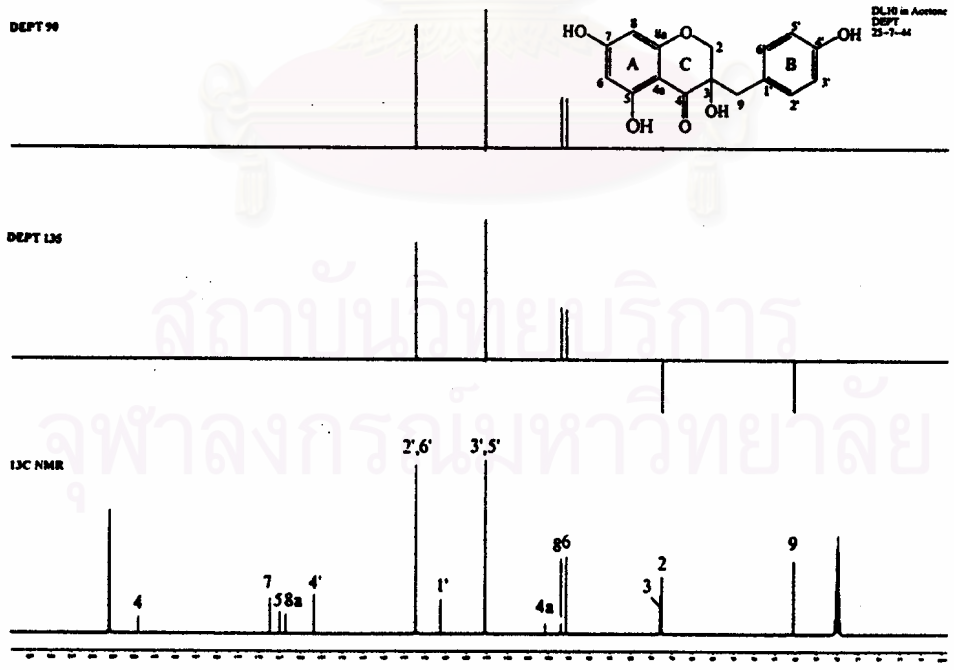

Figure 47 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-G (in acetone-d_6)

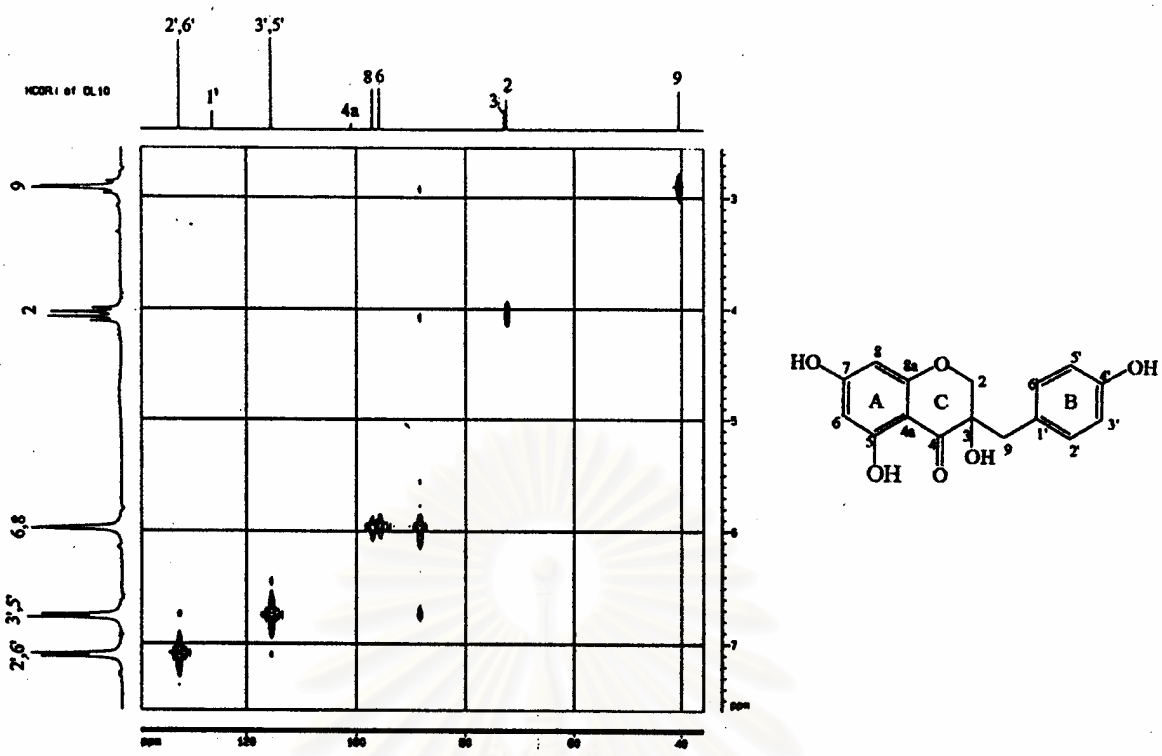

Figure 48a HETCOR spectrum of compound DL-G (in acetone-d_6)

[δ_H 2.6-7.5 ppm, δ_C 38-140 ppm]

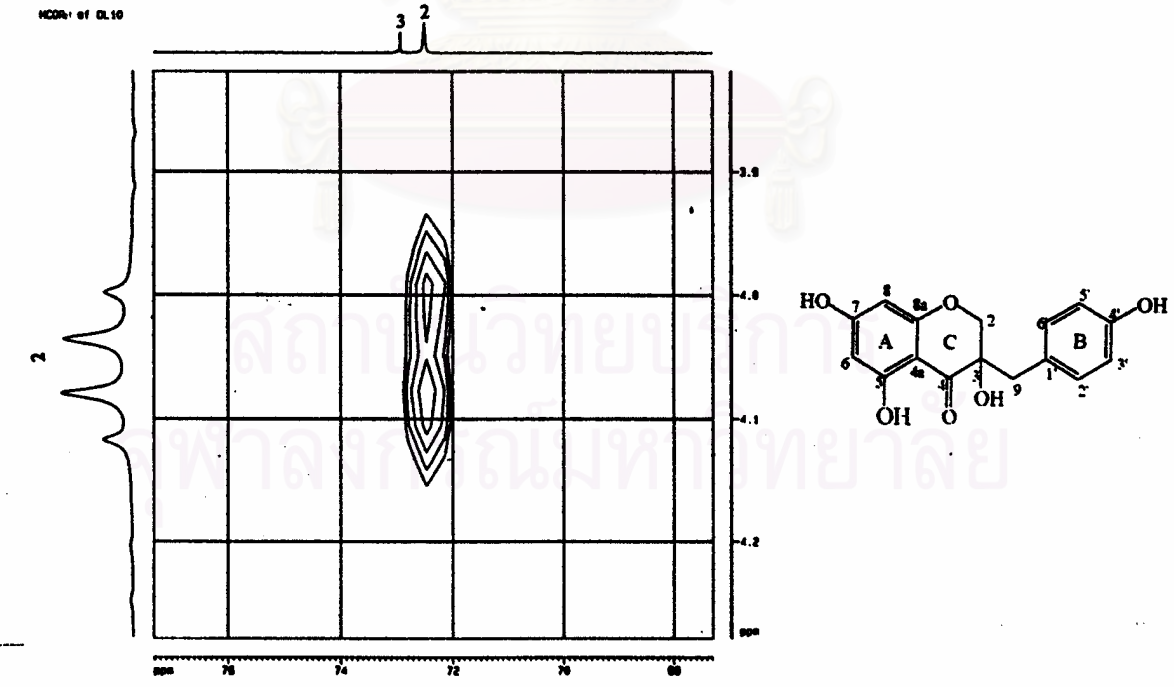

Figure 48b HETCOR spectrum of compound DL-G (in acetone-d_6)

[δ_H 3.8-4.3 ppm, δ_C 68-75 ppm]

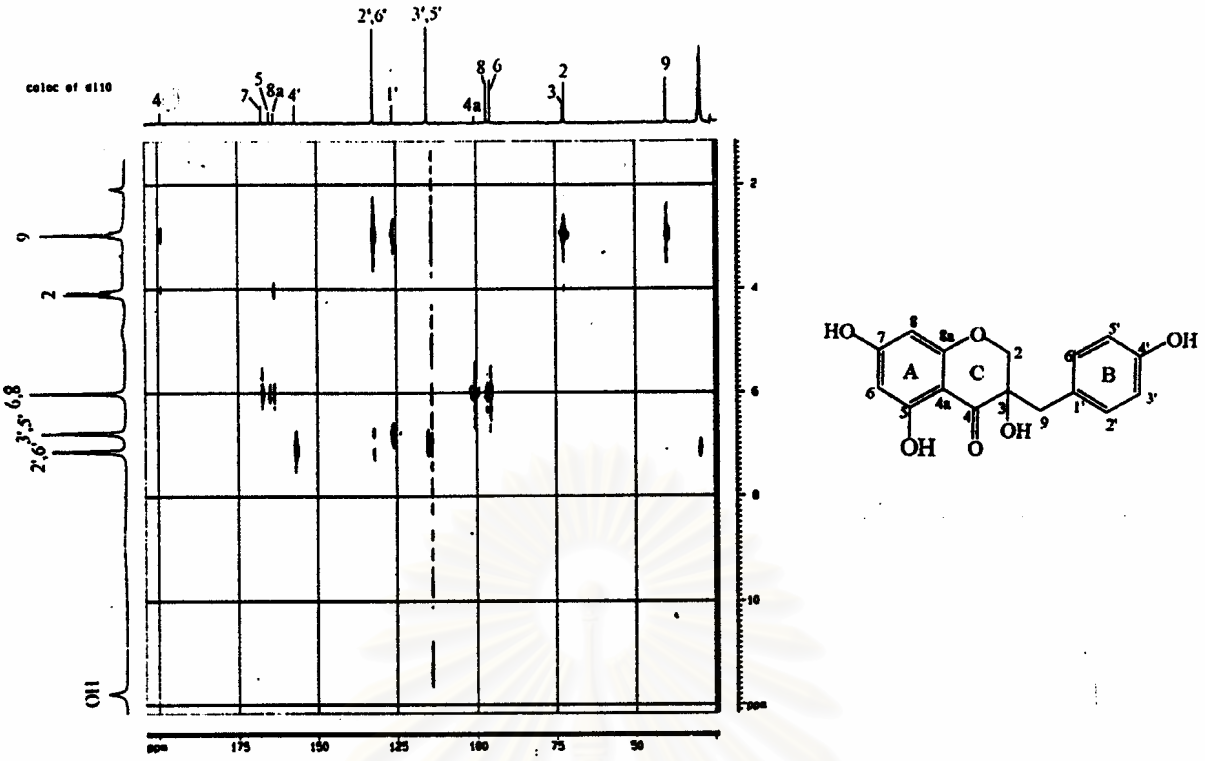

Figure 49 COLOC spectrum of compound DL-G (in acetone-d_6)

[δ_H 1.0-12.0 ppm, δ_C 25-210 ppm]

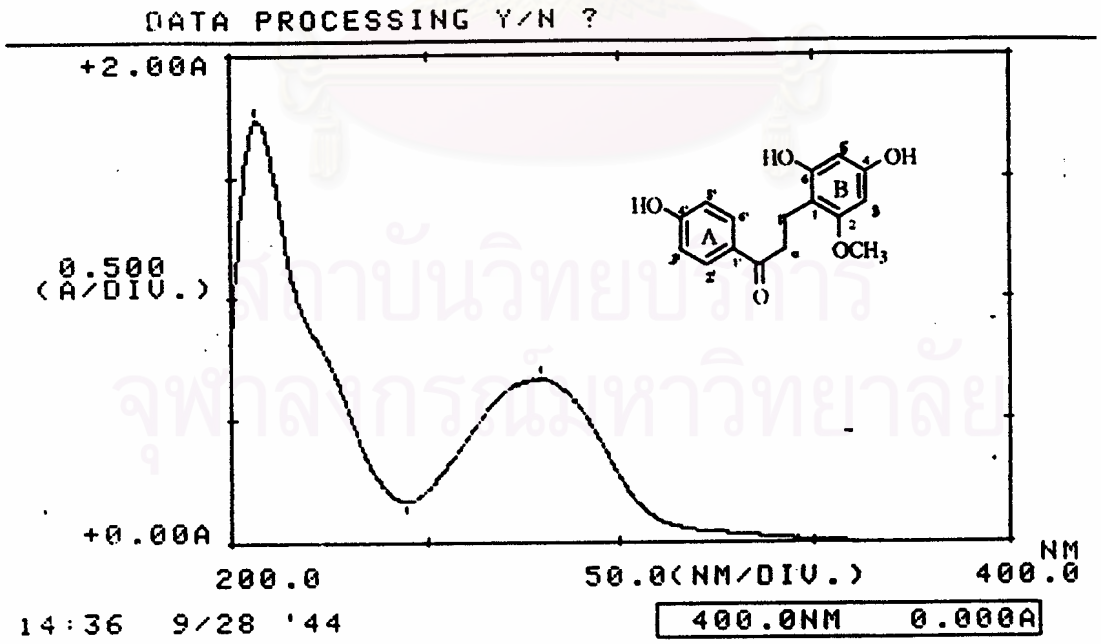

Figure 50 UV spectrum of compound DL-H (in methanol)

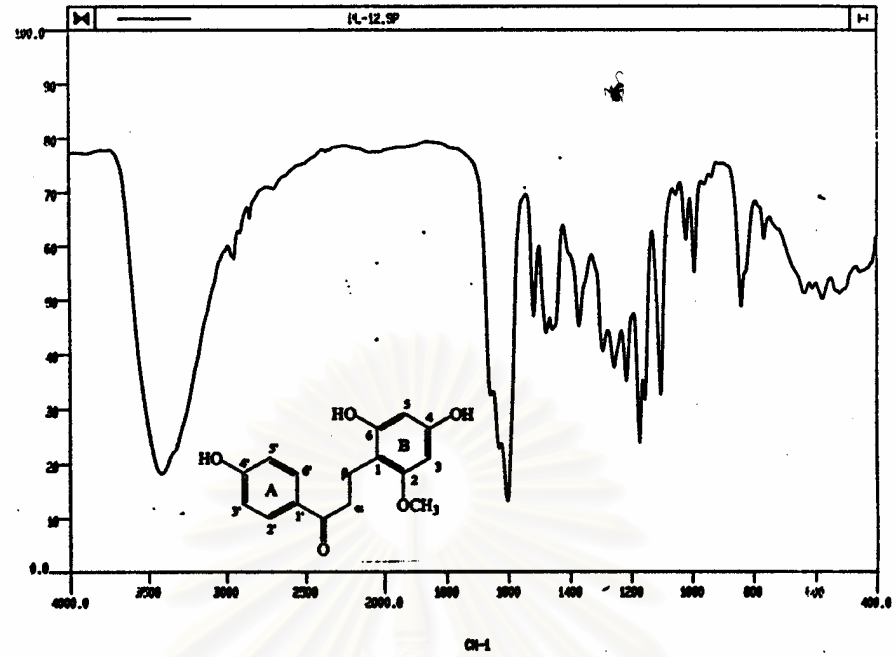

Figure 51 IR spectrum of compound DL-H (KBr disc)

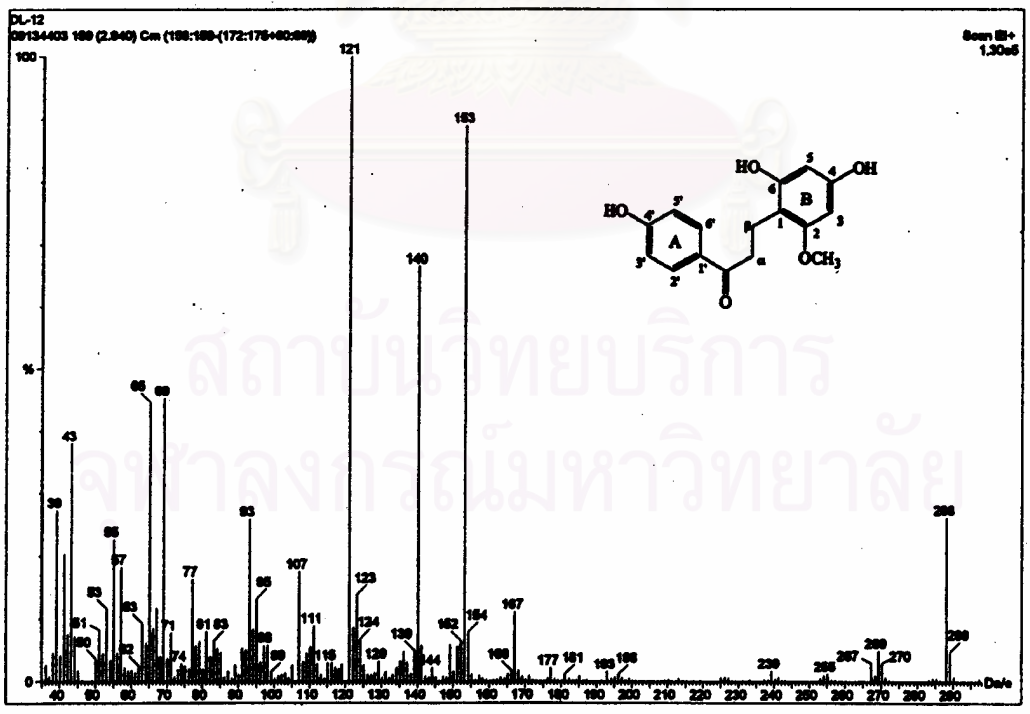

Figure 52 EI mass spectrum of compound DL-H

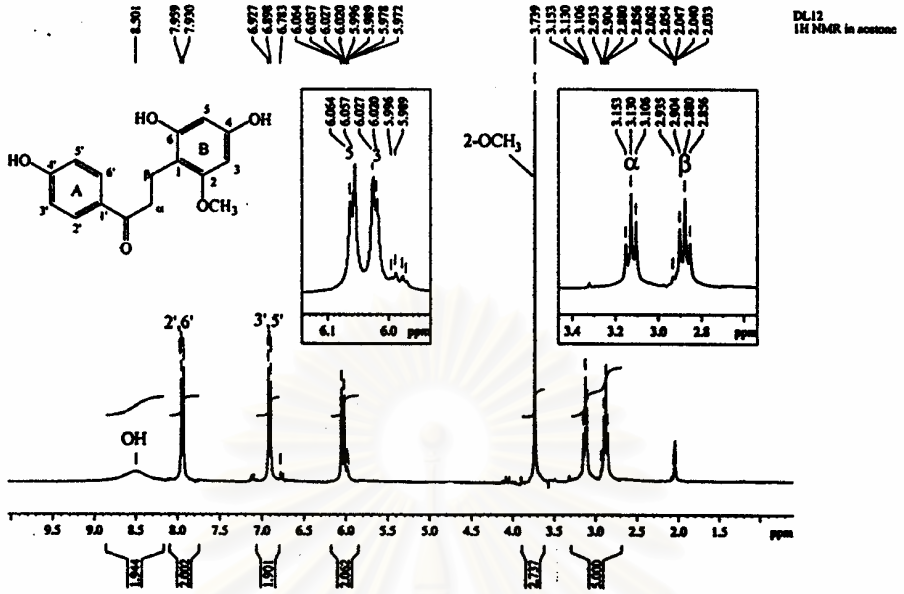

Figure 53 300 MHz ^{1}H NMR spectrum of compound DL-H (in acetone-d_6)

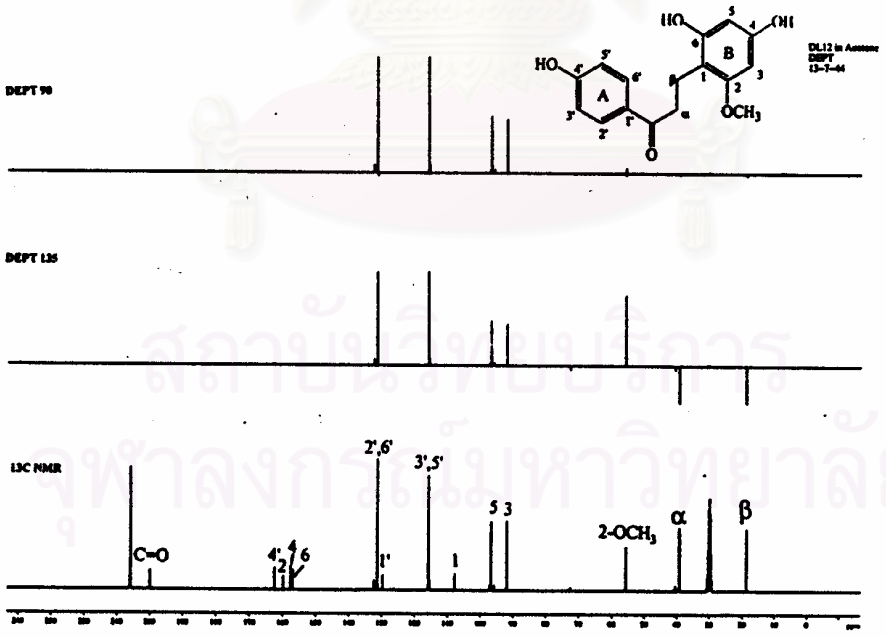

Figure 54 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-H (in acetone-d_6)

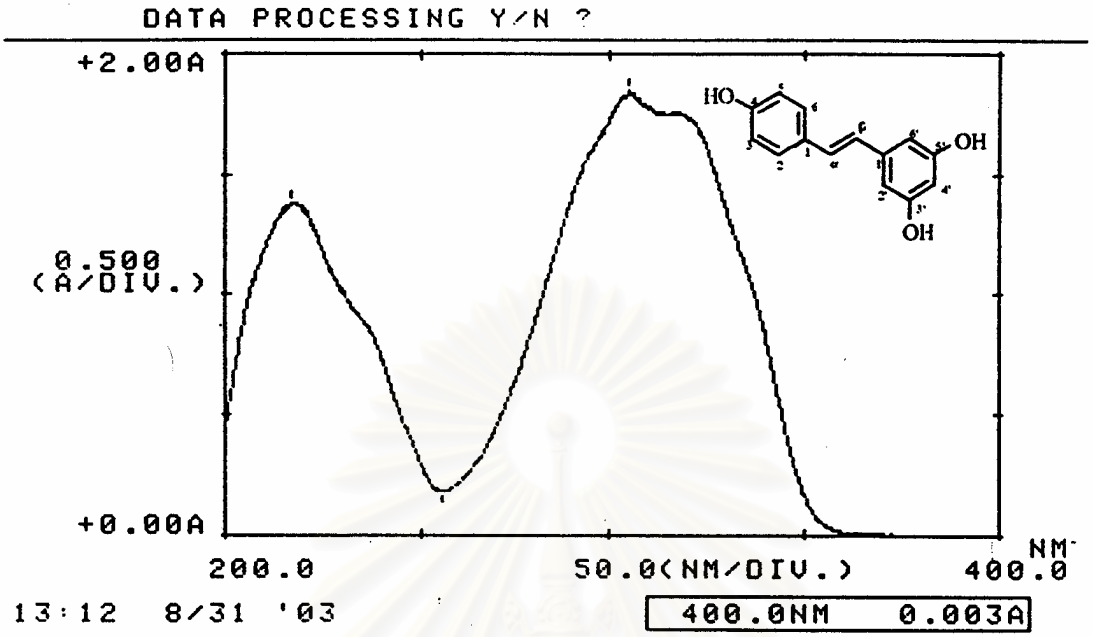

Figure 55 UV spectrum of compound DL-I (in methanol)

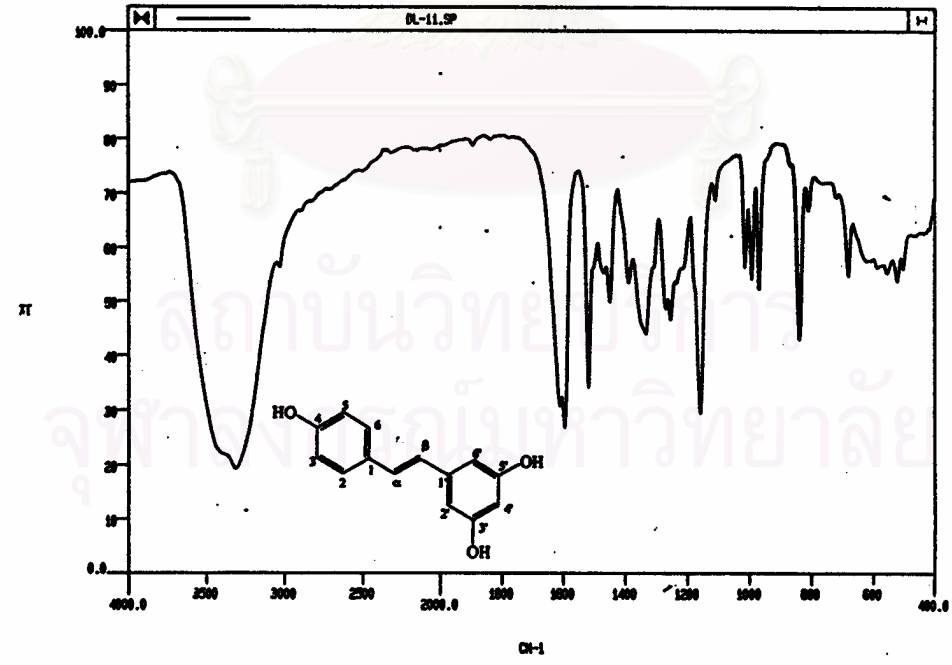

Figure 56 IR spectrum of compound DL-I (KBr disc)

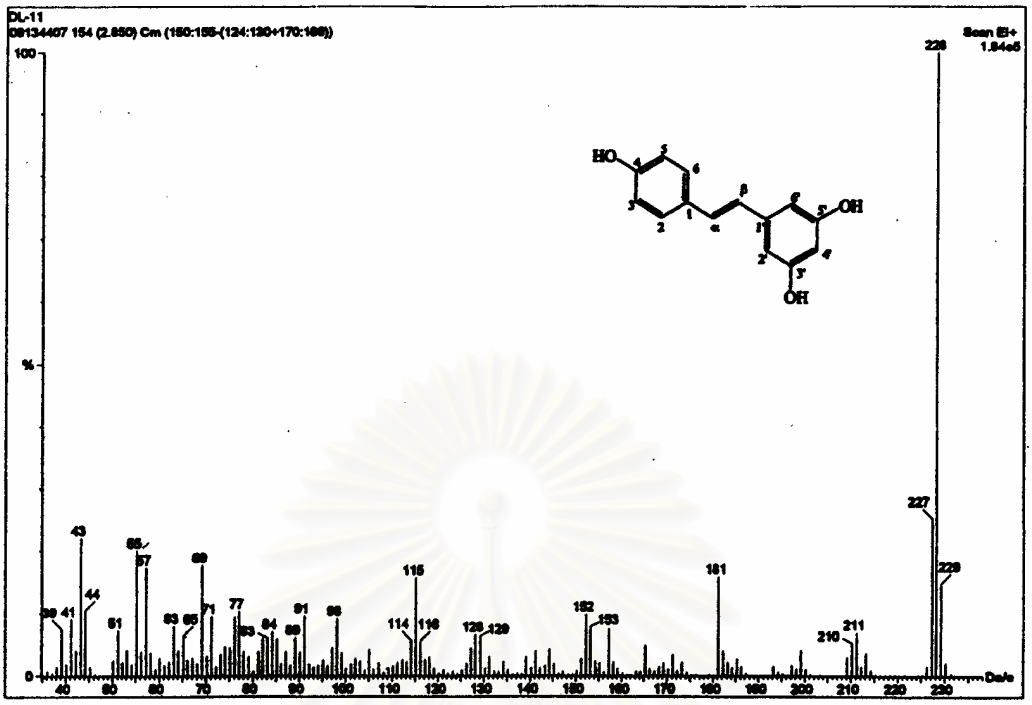

Figure 57 EI mass spectrum of compound DL-I

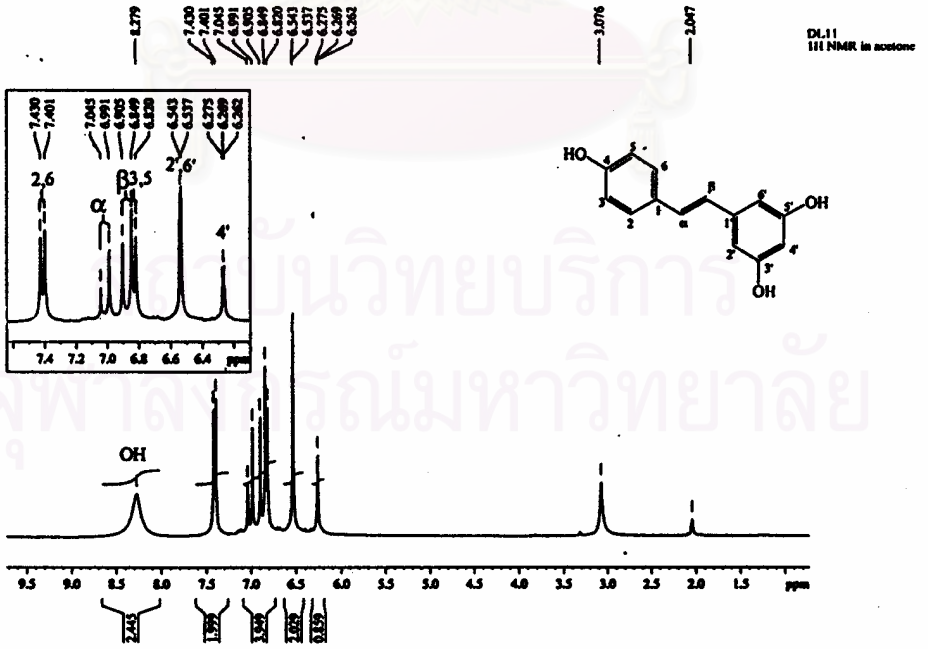

Figure 58 300 MHz ^{1}H NMR spectrum of compound DL-I (in acetone-d_6)

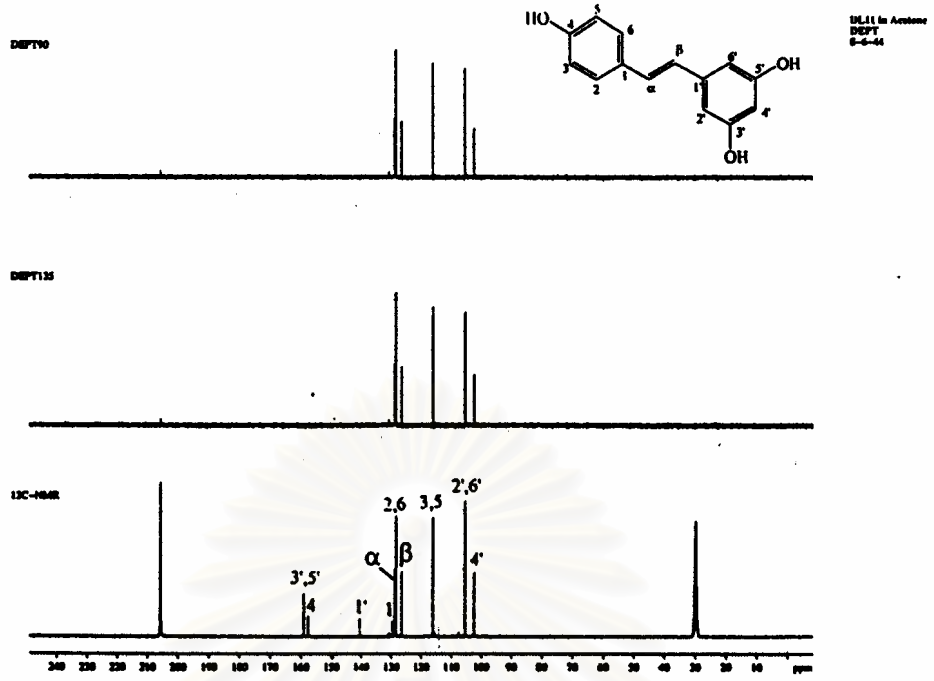

Figure 59 75 MHz ^{13}C NMR, DEPT 90 and DEPT 135 spectra of compound DL-I (in acetone-d_6)

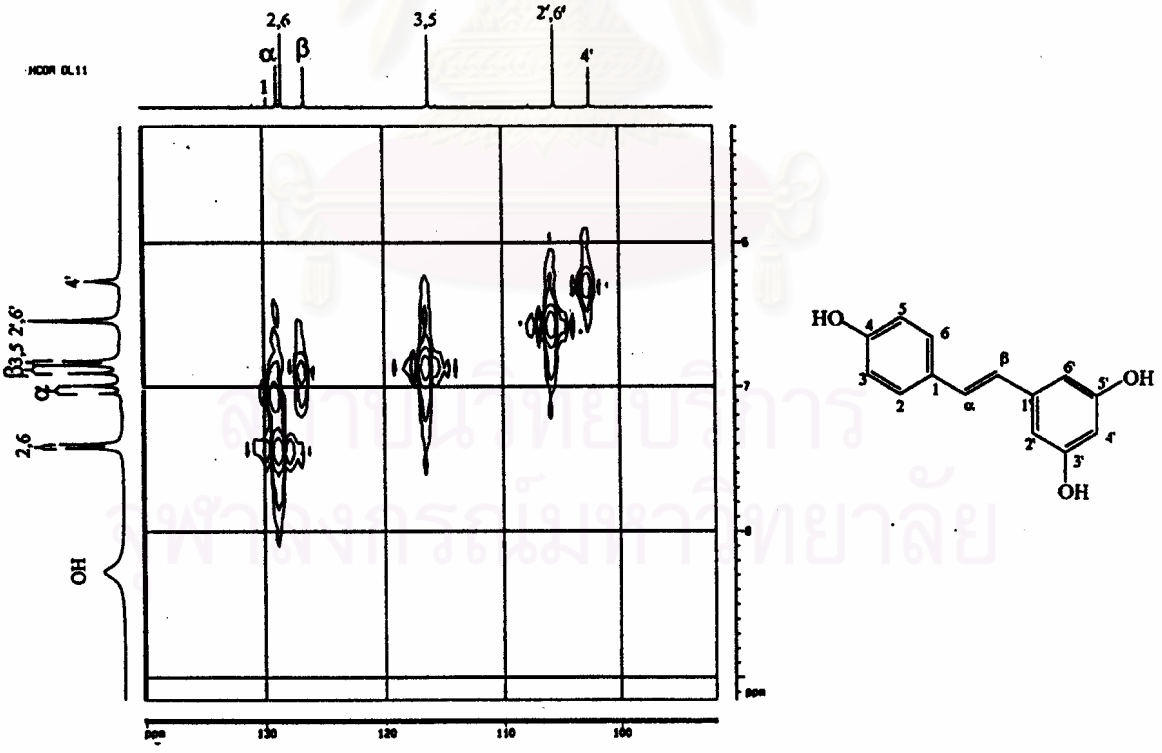

Figure 60 HETCOR spectrum of compound DL-I (in acetone-d_6)

[δ_H 5.0-9.0 ppm, δ_C 90-140 ppm]

VITA

Miss Kanokporn Sawasdee was born on June 7, 1976 in Bangkok, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1999 from the Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University, Thailand.