CHEMICAL CONSTITUENTS OF THE BRANCHES OF *ANOMIANTHUS DULCIS* AND THE BRANCHES OF *DALBERGIA COCHINCHINENSIS* PIERRE

By

Warangkana Pornputtapitak

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

Department of Chemistry

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การศึกษาองค์ประกอบทางเคมีของกิ่งตีนตั่งและกิ่งพะยูง

โดย

นางสาววรางคณา พรพุทธาพิทักษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมีอินทรีย์

ภาควิชาเคมี

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2551

ลิขสิทธิ์ของบัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

The graduate school, Silpakorn University accepted thesis entitled “CHEMICAL CONSTITUENTS OF THE BRANCHES OF *ANOMIANTHUS DULCIS* AND THE BRANCHES OF *DALBERGIA COCHINCHINENSIS* PIERRE” by Warangkana Pornputtapitak in partial fulfillment of the requirements for the degree of master of science, program of organic chemistry.

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49302203 : สาขาวิชาเคมีอินทรีย์

คำสำคัญ : *ANOMIANTHUS DULCIS; DALBERGIA COCHINCHINENSIS* PIERRE; ตีนตั่ง; พะยูง; ANNONACEAE; FABACEAE; LEGUMINOSAE; FABOIDEAE

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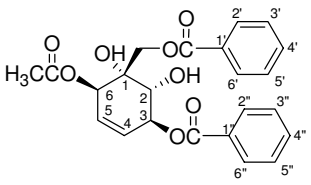
การศึกษาองค์ประกอบทางเคมีของกิ่งตีนตั่ง (*Anomianthus dulcis*) พบสารใหม่ 3 ตัวคืออนุพันธ์ของสารประกอบประเภท polyoxygenated cyclohexene 2 ตัว ได้แก่ (+)-anomianthol A (**2**) และ (+)-anomianthol B (**3**) และ 3-benzoyloxy-4-hydroxybenzaldehyde (**4**) นอกจากนี้ยังพบสารประกอบที่มีรายงานการค้นพบแล้ว อีก 4 ตัวคือ (+)-zeylenol (**1**) syringaldehyde (**5**) 3, 4-dihydroxybenzaldehyde (**6**) และ benzoic acid (**7**)

การศึกษาองค์ประกอบของกิ่งพะยูง (*Dalbergia cochinchinensis* Pierre) พบสารประกอบในกลุ่ม roteniods ได้แก่ 12aα-hydroxyamorphiginin (**9**) ซึ่งเป็นสารใหม่ที่พบในธรรมชาติ และ 12aβ-hydroxyamorphiginin (**8**) นอกจากนี้ยังพบอนุพันธ์ของสารประกอบ benzoic acid ได้แก่ 4-hydroxy-3-methoxybenzoic acid (**12**) และ 4-hydroxybenzoic acid (**13**) และสารประกอบอีก 2 ตัว คือ stigmasta-5, 22-dien-3β-ol-7-one (**10**) และ formononetin (**11**)

พิสูจน์โครงสร้างของสารประกอบดังกล่าวด้วยเทคนิคสเปกโตรสโกปี



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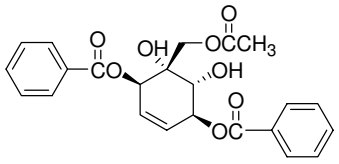

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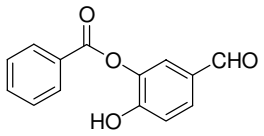

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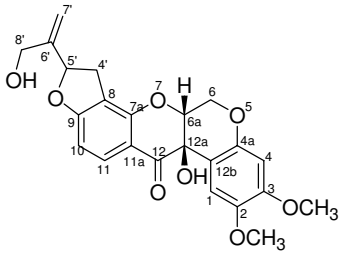

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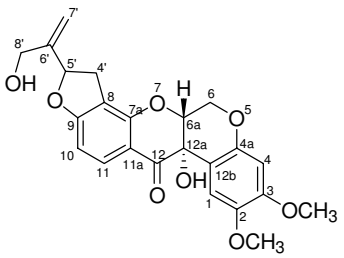

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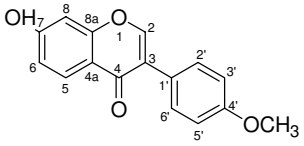

formononetin (**11**)

ลายมือชื่อนักศึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์.....

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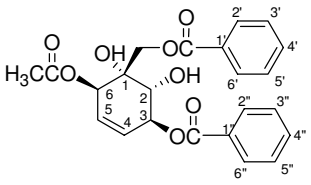
KEY WORDS: *ANOMIANTHUS DULCIS*; *DALBERGIA COCHINCHINENSIS* PIERRE; ANNONACEAE; FABACEAE; LEGUMINOSAE; FABOIDEAE

WARANGKANA PORNPUTTAPITAK: CHEMICAL CONSTITUENTS OF THE BRANCHES OF *ANOMIANTHUS DULCIS* AND THE BRANCHES OF *DALBERGIA COCHINCHINENSIS* PIERRE. THESIS ADVISOR: PROF. PITTAYA TUNTIWACHWUTTIKUL, Ph.D. 102 pp.

Two new polyoxygenated cyclohexene derivatives, (+)-anomianthol A (**2**) and (+)-anomianthol B (**3**) and a new aldehyde, 3-benzoyloxy-4-hydroxybenzaldehyde (**4**) were isolated from the branches of *Anomianthus dulcis* together with four known compounds, (+)-zeylenol (**1**), syringaldehyde (**5**), 3, 4-dihydroxybenzaldehyde (**6**) and benzoic acid (**7**).

A new compound, 12aα-hydroxyamorphiginin (**9**) was isolated from the branches of *Dalbergia cochinchinensis* Pierre together with five known compounds, 12aβ-hydroxyamorphiginin (**8**), stigmasta-5, 22-dien-3β-ol-7-one (**10**), formononetin (**11**), 4-hydroxy-3-methoxybenzoic acid (**12**) and 4-hydroxybenzoic acid (**13**).

The structures were determined on the basis of spectroscopic analysis.



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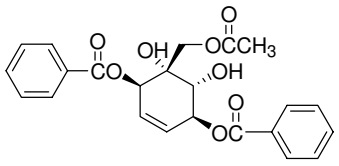

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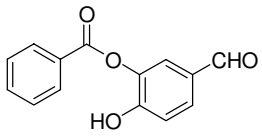
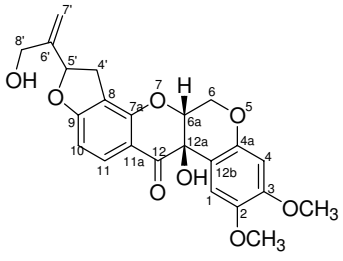
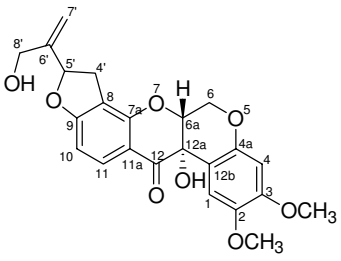


3-benzoyloxy-4-hydroxybenzaldehyde (**4**)



12aβ-hydroxyamorphiginin (**8**)



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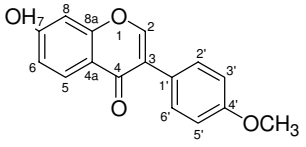

formononetin (**11**)

Department of Chemistry Graduate School, Silpakorn University Academic Year 2008

Student's signature.....

Thesis advisor's signature.....

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CHAPTER 1

CHEMICAL CONSTITUENTS OF THE BRANCHES OF *ANOMIANTHUS DULCIS*

INTRODUCTION

Annonaceae is a large family of aromatic trees, shrubs or climbers (ca 120 genera and more than 2000 species), which are widely distributed in tropical and subtropical regions.

The climber *Anomianthus dulcis* (Dun.) J. Sincliar (Nom Maew Sorn), a monotypic plant in the genus *Anomianthus* is a member of the Annonaceae family that grows in many parts of Southeast Asia.

A. dulcis is a climber, with strong and large vine, creeping up to 4-8 metres distant. Leaves are oval and simple leaf with 10 to 15 cm long and 5-7 cm wide, and leaf apex is acute. The plant produces terminal flower heads, usually pink or pale yellow with soft fragrance. Flowers are 2-4 in clusters or solitary. Each flower is about 3-4 cm in diameter and six-petalled. The fruit is aggregate fruit consists of 8-15 fruits with oval or cylinder 1-1.5 cm long in a bunch. The ripe red fruit is sweet. Each fruit has 1-2 seeded. Normally it flowers in February to May. In Thailand are widely distributed abundantly throughout the Western Forest. Common names are Tob Hoo, Teen Tung Noey, Teen Tung, Nom Maew, Kruer

Kruer Nom Vour and Nom Vour. The synonym is *Rauwenhoffia heterocarpus Zoll*.

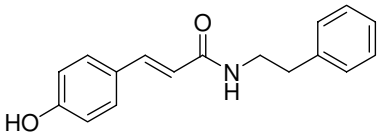
For clinical application, the vine is used for treatment of relapsing fever and the root is used for nourishing milk and treatment of relapsing fever.

Figure 1 *Anomianthus dulcis* (Dun.) J. Sincliar

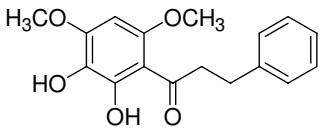
In 1998, Matusch *et al*. [1] reported the isolation of fourteen alkaloids from the methanolic extract of the stem of *Anomianthus dulcis*. The alkaloids, (-)-anonaine, (-)-asimilobine, (-)-anolobine, (-)-roemerine, (+)-stepharine, (-)-reticuline and hordenine, were isolated by preparative HPLC and elucidated by ^{1}H- and ^{13}C-NMR, UV, IR and MS. The alkaloids, *N*-methyllaurotetanine, isoboldine, *N*-nornuciferine, and pronuciferine, were identified by GC-MS. *N*-methyllaurotetanine was comparison with the mass spectrum and retention index whereas isoboldine, *N*-nornuciferine and pronuciferine were comparison of their mass spectral data with those reported previously. Three alkaloids were tentatively identified by GC-MS as discretamine or an isomer, caseamine or an isomer and capaurimine or an isomer. They also reported the isolation of seven alkaloids from the MeOH extract of the leaves of *A. dulcis*. The alkaloids, (-)-anonaine, (-)-asimilobine, (+)-stepharine, pronuciferine, (-)-reticuline, caseamine or an isomer and *N*-methylcrotonosine, were identified by GC-MS.

In addition, Matusch *et al*. [2] isolated squamocin (annonin-1) from the methanolic extract of the branches of *A. dulcis* by preparative HPLC. It was identified by comparison of its spectral data (^{1}H- and ^{13}C-NMR, UV, IR and MS) with those given in the literature. This is the first time to report the isolation of an Annonaceous acetogenin from the genus *Anomianthus*.

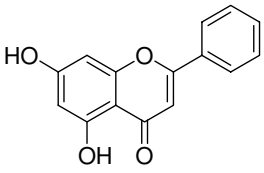
In 1999, Matusch *et al*. [3] reported the isolation of six phenolic compounds, *p*-coumaroyl-β-phenethylamine (**14**), 2', 3'-dihydroxy-4', 6'-dimethoxydihydrochalcone (**15**), chrysin (**16**), pinocembrin (**17**), 5,7-dimethoxy-8-hydroxyflavanone (**18**) and 2', 3'-dihydroxy-4',6'-dimethoxychalcone (**19**) from the methanolic extract of the leaves of *A. dulcis*.



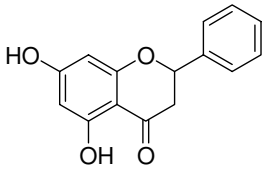
p-coumaroyl-β-phenethylamine (**14**)



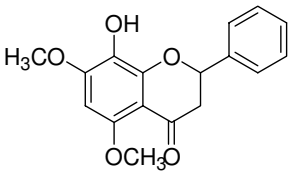
2', 3'-dihydroxy-4',6'-dimethoxydihydrochalcone (**15**)



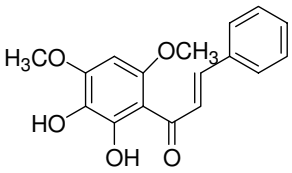
chrysin (**16**)



pinocembrin (**17**)

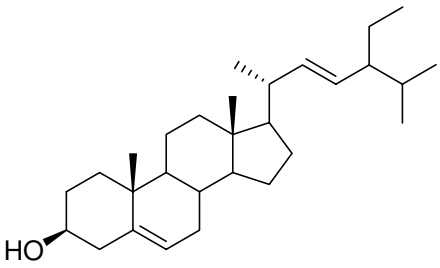


5,7-dimethoxy-8-hydroxyflavanone (**18**)

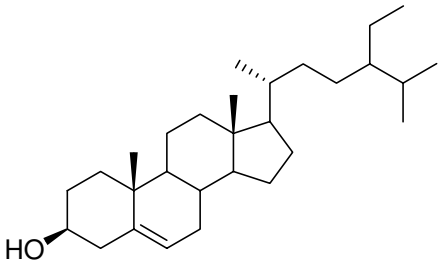


2', 3'-dihydroxy-4',6'-dimethoxychalcone (**19**)

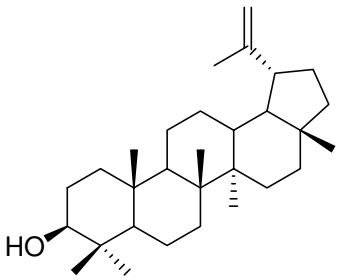
In earlier studies, our group isolated nine compounds, the mixture of stigmasterol (**20**) and β-sitosterol (**21**) (ratio 1:2), lupeol (**22**) [4], the mixture of enone **23** and **24** (ratio 1:2), 5-hydroxy-6, 7-dimethoxyflavanone (**25**) [5], 5-hydroxy-7, 8-dimethoxyflavanone (**26**) [5], flavanone **27** and *N-trans*-feruloyltyramine **28** [6, 7] from EtOAc-soluble fraction of the ethanolic extract of the branches of *A. dulcis.*



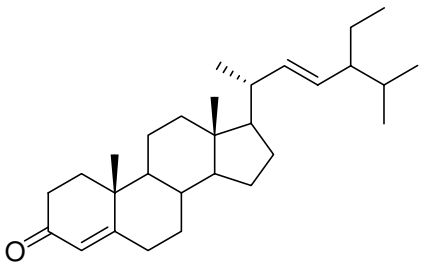
stigmasterol (**20**)



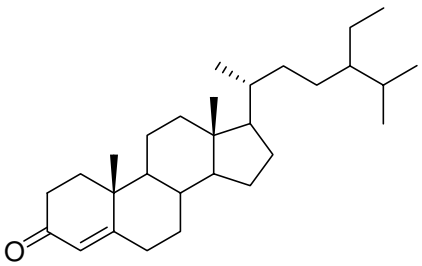
β-sitosterol (**21**)



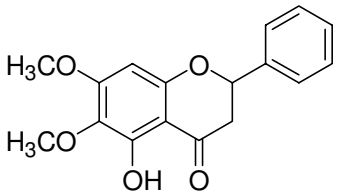
lupeol (**22**)



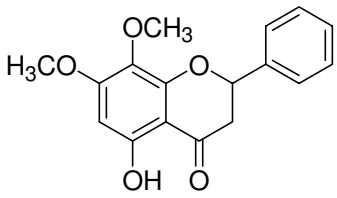
23



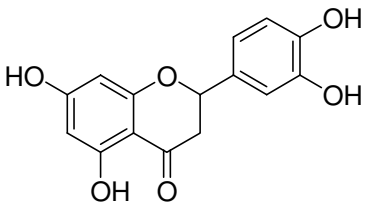
24



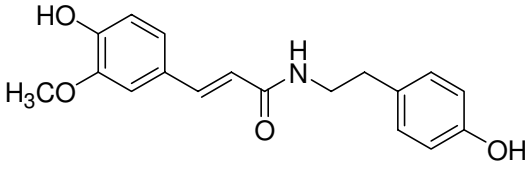
5-hydroxy-6, 7-dimethoxyflavanone (**25**)



5-hydroxy-7, 8-dimethoxyflavanone (**26**)



27



N-trans-feruloyl-tyramine (**28**)

EXPERIMENTAL

Optical rotations were measured in methanol solution with sodium D line (590 nm) on a JASCO P-1010 polarimeter. Ultraviolet spectra (UV) were measured with a Shimadzu UV-240 spectrophotometer. Infrared spectra (IR) were recorded with a JASCO A-302 spectrophotometer. Major bands (ν_{max}) were recorded in wavenumber (cm^{-1}). ^{1}H- and ^{13}C-NMR were measured in $CDCl_3$ or $CDCl_3$-CD_3OD on a Bruker AVANCE 300 (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR) spectrometer. Chemical shifts are in δ (ppm) with tetramethylsilane as an internal standard. Coupling constants (*J*) are given in Hz. The signals in the ^{1}H- and ^{13}C-NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC and HMBC. MS were recorded on a VG 7070 mass spectrometer operating at 70 eV or with a VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectra. HRMS were recorded on a Bruker MicrOTOF mass spectrometer. Column chromatography was carried out using Kieselgel 60 (Merck, 0.063-0.200 mm or 0.015-0.040 mm) and Lichroprep RP-18 (Merck, 40-63 μm). Pre-coated silica gel 60 F_{254} (Merck, layer thickness 0.25 mm) and pre-coated RP-18 F_{254s} (Merck) were used for thin-layer chromatography (TLC) and the compounds were visualized under ultraviolet light or by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. Preparative layer chromatography (PLC) was performed on pre-coated silica gel 60 F_{254} (Merck, 20x20 cm, layer thickness 0.25, 0.50 or 1.00 mm). All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

Plant material

The branches of *Anomianthus dulcis* (Dun.) Sinclair (Annonaceae) were collected from Kaeng Tana National Park, Ubonratchathanee, Thailand, in September 2004. A voucher specimen (SS556/249) has been deposited at the National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Paholyothin Road, Klong 1, Klong Luang, Pathomthani 12120, Thailand.

Extraction and isolation of the branches of *A. dulcis*

The air dried branches of *A. dulcis* (3.66 kg) were extracted with 95% EtOH (3x30.0 l) at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure to give a dark brown foam (200.6 g, AD-1).

AD-1 (a dark brown foam, 200.6 g) was suspended in water (300 ml) and extracted first with EtOAc 3 times (300 ml) and then with *n*-BuOH 3 times (200 ml) in a separatory funnel. The extracts were evaporated under reduced pressure to give a dark brown foam of the EtOAc-soluble extract (34.9 g, AD-1a), a brown foam of the *n*-BuOH-soluble extract (68.8 g, AD-1b) and a brown foam of the H_2O-soluble extract (100.3 g, AD-1c).

AD-1a (34.9 g) was separated by flash column chromatography using silica gel 60 [Merck, 0.015-0.040 mm, 240.0 g, diameter x height (13.0 cm x 5.0 cm)]. The column was eluted with 500 ml each fraction of hexane, gradient of EtOAc/hexane, and gradient of MeOH/EtOAc and were evaporated under reduced

pressure to give 17 fractions (Table 1). Bioactivities of each fraction were investigated. [Table 2 for anti-cancer (KB and BC cells) and Table 3 for anti-TB and anti-malarial]

Table 1 Fractions obtained from AD-1a.

Fraction No.	Eluent	Weight (g)	Physical characteristic
AD-1a-1 AD-1a-2	hexane	0.34	a colorless oil
AD-1a-3	10 % EtOAc/ hexane	0.20	a yellow oil
AD-1a-4	20 % EtOAc/ hexane	2.26	a brown wax
*AD-1a-5	30 % EtOAc/ hexane	3.50	a green oil with solid
AD-1a-6	40 % EtOAc/ hexane	1.43	a green oil with solid
*AD-1a-7	50 % EtOAc/ hexane	1.12	a brown wax
**AD-1a-8	60 % EtOAc/ hexane	0.90	a brown oil
**AD-1a-9	70 % EtOAc/ hexane	0.51	a brown oil
AD-1a-10	80 % EtOAc/ hexane	0.88	a green wax
*AD-1a-11	90 % EtOAc/ hexane	2.79	a dark green foam
AD-1a-12	EtOAc	2.44	a pale green foam
AD-1a-13	2 % MeOH/ EtOAc	2.11	a pale brown foam
AD-1a-14	5 % MeOH/ EtOAc	1.54	a brown foam
AD-1a-15	10 % MeOH/ EtOAc	2.63	a brown foam
AD-1a-16	20 % MeOH/ EtOAc	4.37	a dark brown foam
AD-1a-17	40 % MeOH/ EtOAc	2.61	a brown foam

* Fractions were already investigated previously.

** Fractions were further investigated.

Table 2 Bioactivities of fractions obtained from AD-1a.

Fraction	Anti-cancer (KB)	ED_{50} (μg/mL)	Anti-cancer (BC)	ED_{50} (μg/mL)
AD-1a-1	Moderately active	4.59	Moderately active	9.37
AD-1a-2	Weakly active	15.95	Weakly active	18.15
AD-1a-3	Inactive	-	Inactive	-
AD-1a-4	Inactive	-	Inactive	-
AD-1a-5	-	-	-	-
AD-1a-6	Moderately active	3.41	Moderately active	5.56
AD-1a-7	Strongly active	0.70	Strongly active	0.97
AD-1a-8	Strongly active	0.22	Strongly active	0.20
AD-1a-9	Strongly active	0.34	Strongly active	0.32
AD-1a-10	Strongly active	0.13	Strongly active	0.17
AD-1a-11	Waiting	-	Waiting	-
AD-1a-12	Waiting	-	Waiting	-
AD-1a-13	Waiting	-	Waiting	-
AD-1a-14	Waiting	-	Waiting	-
AD-1a-15	Waiting	-	Waiting	-
AD-1a-16	Waiting	-	Waiting	-
AD-1a-17	Waiting	-	Waiting	-

Table 3 Bioactivities of fractions obtained from AD-1a.

Fraction	Anti TB	ED_{50} (μg/mL)	Anti-malarial	ED_{50} (μg/mL)
AD-1a-1	Inactive	-	Inactive	-
AD-1a-2	Inactive	-	Inactive	-
AD-1a-3	Active	200	Inactive	-
AD-1a-4	Active	200	Inactive	-
AD-1a-5	-	-	-	-
AD-1a-6	Waiting	-	Inactive	-
AD-1a-7	Waiting	-	Inactive	-
AD-1a-8	Active	100	Inactive	-
AD-1a-9	Active	100	Inactive	-
AD-1a-10	Active	100	Inactive	-
AD-1a-11	Active	100	Inactive	-
AD-1a-12	Active	100	Inactive	-
AD-1a-13	Active	200	Inactive	-
AD-1a-14	Active	200	Inactive	-
AD-1a-15	Active	200	Inactive	-
AD-1a-16	Active	200	Inactive	-
AD-1a-17	Inactive	-	Inactive	-

Fraction AD-1a-8

Fraction AD-1a-8 (a brown oil, 900 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 80 g) using hexane/EtOAc (3:1, 2.5:1, 2:1, 1.5:1, 1:1, 1:2, 1:3, 1:4), EtOAc and MeOH as the eluent. The fractions obtained were combined on the basis of their behaviors on TLC and evaporated under reduced pressure to give 20 fractions. (Table 4)

Table 4 Fractions obtained from AD-1a-8.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-1	10.7	a colorless semisolid
AD-1a-8-2	9.0	a yellow oil
AD-1a-8-3	2.3	a yellow solid
AD-1a-8-4	3.8	a brown semisolid
AD-1a-8-5	3.5	a green solid
AD-1a-8-6	5.7	a pale brown solid
AD-1a-8-7	15.4	a green solid
AD-1a-8-8	16.9	a green solid
*AD-1a-8-9	42.0	a brown solid
AD-1a-8-10	14.0	a red solid
*AD-1a-8-11	100.1	a brown solid
*AD-1a-8-12	89.1	a brown solid
*AD-1a-8-13	38.7	a brown solid
*AD-1a-8-14	77.5	a brown wax
AD-1a-8-15	36.2	a brown wax
AD-1a-8-16	21.8	a brown wax
AD-1a-8-17	12.3	a brown wax
AD-1a-8-18	15.3	a brown wax
AD-1a-8-19	167.2	a dark brown solid
AD-1a-8-20	20.6	a dark brown solid

* Fractions were further investigated.

Fraction AD-1a-8-9 (a brown solid, 42.0 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 2 plates) using benzene/EtOAc (10:1; 2 runs, 5:1; 2 runs) as the developing solvent to give 3 fractions. (Table 5)

Table 5 Fractions obtained from AD-1a-8-9.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-9-1	2.7	a orange solid
*AD-1a-8-9-2	17.4	a brown solid
*AD-1a-8-9-3	12.7	a yellow solid

* Fractions were further investigated.

Fraction AD-1a-8-9-2 (a brown solid, 17.4 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 1 plate) using CH_2Cl_2/ MeOH/H_2O (50:3:1; 2 runs) as the developing solvent to give 2 fractions. (Table 6)

Table 6 Fractions obtained from AD-1a-8-9-2.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-9-2-1	1.0	a white solid
AD-1a-8-9-2-2	8.2	a pale brown solid

AD-1a-8-9-2-2 (8.2 mg) were identified as compound **A-1** (partially pure).

Fraction AD-1a-8-9-3 (a yellow solid, 12.7 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 1 plate) using CH_2Cl_2/MeOH/H_2O (80:3:1; 3 runs) as the developing solvent to give 2 fractions. (Table 7)

Table 7 Fractions obtained from AD-1a-8-9-3.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-9-3-1	9.1	a colorless solid
AD-1a-8-9-3-2	2.9	a pale yellow solid

AD-1a-8-9-3-1 (9.1 mg) was identified as compound **A-2**.

AD-1a-8-9-3-2 (2.9 mg) was identified as compound **A-1** (partially pure).

Fraction AD-1a-8-11 (a brown solid, 100.1 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 15 g) using CH_2Cl_2/MeOH/H_2O (300:3:1, 250:3:1, 200:3:1) as the eluent to give 9 fractions. (Table 8)

Table 8 Fractions obtained from AD-1a-8-11.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-11-1	8.1	a brown oil
AD-1a-8-11-2	2.1	a brown oil
AD-1a-8-11-3	20.5	a yellow oil
AD-1a-8-11-4	9.5	a pale brown foam
*AD-1a-8-11-5	14.6	a pale brown foam
AD-1a-8-11-6	4.9	a pale yellow solid
AD-1a-8-11-7	4.1	a brown solid + white crystals
AD-1a-8-11-8	2.9	a pale yellow solid
AD-1a-8-11-9	4.0	a pale yellow solid

* Fractions were further investigated.

AD-1a-8-11-4 (9.5 mg) was identified as compound **A-3** (partially pure).

AD-1a-8-11-9 (4.0 mg) was identified as compound **A-4**.

Fraction AD-1a-8-11-5 (a brown foam, 14.6 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.25 mm, 2 plates) using CH_2Cl_2/MeOH/H_2O (50:3:1; 1 run) as the developing solvent to give **A-3** as a pale yellow resin (9.1 mg).

Fraction AD-1a-8-12 (a brown solid, 89.1 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 15 g) using CH_2Cl_2/MeOH/H_2O (300:3:1, 250:3:1, 200:3:1, 150:3:1) as the eluent to give 8 fractions. (Table 9)

Table 9 Fractions obtained from AD-1a-8-12.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-12-1	1.4	a pale brown solid
AD-1a-8-12-2	7.2	a brown wax
AD-1a-8-12-3	17.0	a yellow oil
AD-1a-8-12-4	13.6	a pale yellow resin
AD-1a-8-12-5	15.3	a yellow oil + solid
AD-1a-8-12-6	8.3	a pale yellow solid
AD-1a-8-12-7	1.9	a orange solid + colorless crystals
AD-1a-8-12-8	0.4	a pale yellow oil

AD-1a-8-12-1 (1.4 mg) was identified as compound **A-5**.

AD-1a-8-12-4 (13.6 mg) was identified as compound **A-3**.

Fraction AD-1a-8-13 (a brown solid, 38.7 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 1.00 mm, 1 plate) using CH_2Cl_2/ MeOH/H_2O (50:3:1; 1 run) and hexane/EtOAc (2:1; 2 runs) as the developing solvent to give 7 fractions. (Table 10)

Table 10 Fractions obtained from AD-1a-8-13.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-13-1	1.5	a pale brown solid
AD-1a-8-13-2	6.0	a brown solid
AD-1a-8-13-3	10.1	a brown semisolid
AD-1a-8-13-4	8.7	a brown solid
AD-1a-8-13-5	5.4	a dark brown solid
AD-1a-8-13-6	3.6	a pale brown solid
AD-1a-8-13-7	1.5	a brown solid

AD-1a-8-13-6 (3.6 mg) was identified as compound **A-6** (not pure).

Fraction AD-1a-8-14 (a brown wax, 77.5 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 1.00 mm, 2 plates) using CH_2Cl_2/MeOH/H_2O (50:3:1; 2 runs) and hexane/EtOAc (2:1; 3 runs) as the developing solvent to give 5 fractions. (Table 11)

Table 11 Fractions obtained from AD-1a-8-14.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-14-1	2.1	a yellow oil + white crystals
AD-1a-8-14-2	26.9	a yellow semisolid
AD-1a-8-14-3	6.8	a yellow solid
*AD-1a-8-14-4	32.2	a yellow solid
AD-1a-8-14-5	4.4	a yellow solid

* Fractions were further investigated.

Fraction AD-1a-8-14-4 (a yellow solid, 32.2 mg) was purified by preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 2 plates) using CH_2Cl_2/MeOH/H_2O (50:3:1; 1 run) as the developing solvent to give AD-1a-8-14-4-P as a pale yellow solid (22.3 mg).

Fraction AD-1a-8-14-4-P (a pale yellow solid, 22.3 mg) was further purified on a column of Lichroprep RP-18 (Merck, 40-63 μm, 6.0 g) and eluted with MeOH/H_2O (4:1) to give 4 fractions. (Table 12)

Table 12 Fractions obtained from AD-1a-8-14-4-P.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-8-14-4-P-1	11.5	a yellow oil
AD-1a-8-14-4-P-2	1.6	a yellow oil
AD-1a-8-14-4-P-3	1.6	colorless needles
AD-1a-8-14-4-P-4	2.6	a yellow oil

AD-1a-8-14-4-P-3 (1.6 mg) was identified as compound **A-6**.

Fractions AD-1a-9

Fractions AD-1a-9 (a brown oil, 0.51 g) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 51 g) using CH_2Cl_2/MeOH/H_2O (100:3:1; 50:3:1. 30:3:1) as the eluent to give 11 fractions. (Table 13)

Table 13 Fractions obtained from AD-1a-9.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-9-1	14.7	a brown wax
AD-1a-9-2	47.4	a brown wax
AD-1a-9-3	161.7	a brown wax
AD-1a-9-4	171.1	a brown wax
AD-1a-9-5	75.9	a brown wax
*AD-1a-9-6	38. 0	a brown wax
AD-1a-9-7	49.4	a brown wax
AD-1a-9-8	15.7	a brown wax
AD-1a-9-9	22.4	a brown wax
AD-1a-9-10	24.9	a brown wax
AD-1a-9-11	15.4	a brown wax

* Fractions were further investigated.

Fraction AD-1a-9-6 (a brown wax, 38.0 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 2 plates) using CH_2Cl_2/MeOH/H_2O (20:3:1; 1 run) as the developing solvent to give 4 fractions. (Table 14)

Table 14 Fractions obtained from AD-1a-9-6.

Fraction No.	Weight (mg)	Physical characteristic
* AD-1a-9-6-1	10.6	a pale yellow solid
AD-1a-9-6-2	11.5	a pale brown solid
AD-1a-9-6-3	7.6	a pale brown solid
AD-1a-9-6-4	2.7	a pale yellow solid

* Fractions were further investigated.

AD-1a-9-6-2 (11.5 mg) was identified as compound **A-7** (partially pure).

Fraction AD-1a-9-6-1 (a pale yellow solid, 10.6 mg) was further purified on a column of Lichroprep RP-18 (Merck, 40-63 μm, 6.0 g) and eluted with MeOH/H_2O (4:1) to give 3 fractions. (Table 15)

Table 15 Fractions obtained from AD-1a-9-6-1.

Fraction No.	Weight (mg)	Physical characteristic
AD-1a-9-6-1-1	3.7	colorless needles
AD-1a-9-6-1-2	0.9	a yellow solid
AD-1a-9-6-1-3	1.4	a white solid

AD-1a-9-6-1-1 (3.7 mg) was identified as compound **A-6**.

A-1

A-1 was obtained as a white solid; m.p. 121.0 – 123.5 °C; ^{1}H–NMR ($CDCl_3$): see **Table 16**.

A-2

A-2 was obtained as a colorless solid; m.p. 119-120 °C; $[\alpha]_D^{26}$+ 100.0° (*c* = 0.02, MeOH); UV λ_{max}^{MeOH} (log ε) nm : 201 (4.38), 230 (4.40), 273(3.29), 280(3.21); IR ν_{max}^{nujol}cm^{-1} : 3450, 2923, 1693, 1299, 1170, 1113, 1069, 1024, 967, 721; HRTOFMS found : *m/z* 427.1393 $[M+H]^+$; Calculated for $[C_{23}H_{22}O_8+H]^+$: *m/z* 427.1391; EI MS *m/z* (relative intensity, %) : 427 $[M+H]^+$ (1%), 409(2), 367(2), 337(1), 304(2), 273(2), 231(3), 215(4), 203(3), 190(2), 182(2), 163(12), 153(2), 141(13), 122(14) 105(100), 99(14), 77(88); ^{1}H–NMR ($CDCl_3$) : see **Table 18**; ^{13}C–NMR ($CDCl_3$) : see **Table 19**.

A-3

A-3 was obtained as a pale yellow resin; $[\alpha]_D^{26}$+ 76.1° (*c* = 0.02, MeOH); UV λ_{max}^{MeOH} (log ε) nm : 201(4.39), 230 (4.41), 274(3.25), 281(3.16); IR ν_{max}^{nujol}cm^{-1} : 3450, 2923, 1715, 1269, 1170, 1069, 1024, 956, 721; HRTOFMS found : *m/z* 449.1215 $[M+Na]^+$; Calculated for $[C_{23}H_{22}O_8+H]^+$: *m/z* 449.1212; EI MS *m/z* (relative intensity, %) : 427 $[M+Na]^+$ (2%), 409(12), 367(1), 335(4), 305(20), 275(7), 231(12), 226(8), 203(33), 190(2), 182(4), 163(20), 153(20), 141(12), 122(47) 105(100), 99(17), 77(99); ^{1}H–NMR ($CDCl_3$) : see **Table 18**; ^{13}C–NMR ($CDCl_3$) : see **Table 19**.

A-4

A-4 was obtained as colorless needles; m.p. 88-92 °C; UV λ_{max}^{MeOH} (log ε) nm : 201(4.33), 227 (4.23), 278(3.75); IR ν_{max}^{nujol} cm^{-1} : 3166, 2925, 2726, 1697, 1586, 1288, 1169, 1071, 1026, 936, 721, 663; ^{1}H–NMR ($CDCl_3$), ^{13}C–NMR ($CDCl_3$) : see **Table 20**.

A-5

A-5 was obtained as a pale brown solid; m.p. 110.0 – 113.0 °C; ^{1}H–NMR ($CDCl_3$): see **Table 17**.

A-6

A-6 was obtained as colorless needles; m.p. 132-134 °C; $[\alpha]_D^{26}$+ 121.9° (*c* = 0.02, MeOH); UV λ_{max}^{MeOH} (log ε) nm : 201 (4.38), 230 (4.40), 273 (3.54); IR ν_{max}^{nujol} cm^{-1} : 3451, 2923, 1694, 1284, 1170, 1121, 1072, 952, 721; ^{1}H–NMR ($CDCl_3$), ^{13}C–NMR ($CDCl_3$) : see **Table 21**.

A-7

A-7 was obtained as a pale brown solid; m.p. 150.0 – 155.0 °C; ^{1}H–NMR ($CDCl_3$): see **Table 17**.

Table 16 ^{1}H -NMR spectral data of **A-1**.

position	**A-1** [benzoic acid, **7**]
1	-
2, 6	7.99 (2H, dd, 1.1, 7.2)
3, 5	7.28 (2H, t, 7.2)
4	7.45 (1H, tt, 1.1, 7.2)

Table 17 ^{1}H-NMR spectral data of **A-5** and **A-7**.

position	**A-5** [syringaldehyde, **5**]	**A-7** [3, 4-dihydroxybenzaldehyde, **6**]
1	-	-
2	7.18 (1H, s)	7.28 (1H, d, 1.7)
3	-	-
4	-	-
5	-	6.90 (1H, d, 7.8)
6	7.18 (1H, s)	7.25 (1H, dd, 1.7, 7.8)
CH_3O	4.00 (6H, s)	-
CHO	9.84 (1H, s)	9.65 (1H, s)

Table 18 ^{1}H-NMR spectral data of **A-2** and **A-3**.

position	**A-2** [(+)- anomianthol A, **2**]	**A-3** [(+)- anomianthol B, **3**]
1	-	-
2	4.26 (1H, d, 6.5)	4.27 (1H, d, 6.1)
3	5.78 (1H, dd, 1.7, 6.5)	5.79 (1H, ddd, 1.4, 2.5, 6.1)
4	5.93 (1H, d, 1.7)	6.01 (1H, dd, 2.5, 10.2)
5	5.93 (1H, d, 1.7)	6.08 (1H, ddd, 1.4, 3.6, 10.2)
6	5.56 (1H, d, 1.7)	5.70 (1H, d, 3.6)
7	4.58 (1H, d, 12.0), 4.82 (1H, d, 12.0)	4.43 (1H, d, 12.3), 4.64 (1H, d, 12.3)
1', 1"	-	-
2', 6' or 2", 6"	7.95 (2H, dd, 1.3, 7.8)	8.06 (2H, dd, 1.4, 7.8)
2", 6" or 2', 6'	8.03 (2H, dd, 1.3, 7.8)	8.10 (2H, dd, 1.4, 7.8)
3', 5' or 3", 5"	-	7.48 (2H, t, 7.8)
3", 5" or 3', 5'	-	7.49 (2H, t, 7.8)
3', 5', 3", 5"	7.40 (4H, dt, 1.3, 7.8)	-
4', 4"	7.55 (2H, dt, 1.3, 7.8)	7.62 (2H, dt, 1.4, 7.8)
$\underline{CH_3}CO$	2.10 (3H, s)	1.90 (3H, s)
$CH_3\underline{CO}$	-	-
Ar$\underline{CO}$	-	-
3-O$\underline{CO}$Ar	-	-
7-O$\underline{CO}$Ar	-	-

Table 19 ^{13}C-NMR spectral data of **A-2** and **A-3**.

position	**A-2** [(+)- anomianthol A, **2**]	**A-3** [(+)- anomianthol B, **3**]
1	74.5	74.5
2	71.1	71.2
3	73.4	73.3
4	128.6	128.8
5	126.6	126.6
6	70.6	71.1
7	66.4	66.2
1', 1"	129.4 (2X)	129.5 (2X)
2', 6' or 2", 6"	129.6 (2X)	129.7 (2X)
2", 6" or 2', 6'	129.8 (2X)	129.8 (2X)
3', 5' or 3", 5"	-	128.5 (2X)
3", 5" or 3', 5'	-	128.6 (2X)
3', 5', 3", 5"	128.4 (4X)	-
4', 4"	133.3, 133.4	133.5 (2X)
$\underline{CH_3}CO$	20.9	20.5
$CH_3\underline{CO}$	170.2	171.6
Ar$\underline{CO}$	167.0 (2X)	-
3-O$\underline{CO}$Ar	-	166.9
7-O$\underline{CO}$Ar	-	165.7

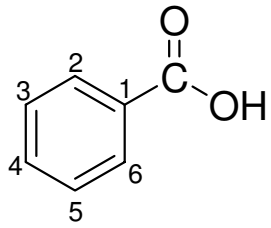
Table 20 ^{1}H- and ^{13}C-NMR spectral data of **A-4**.

(3-benzoyloxy-4-hydroxybenzaldehyde, **4**)

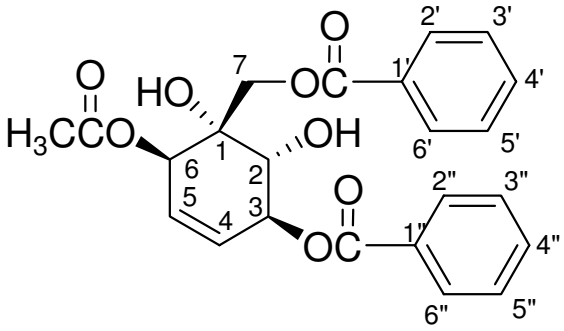
position	δ_H	δ_C
1	-	129.3
1-CHO	9.66 (1H, s)	191.9
2	7.27 (1H, d, 1.7)	113.9
3	-	145.2
4	-	152.0
5	6.89 (1H, d, 7.8)	114.9
6	7.25 (1H, dd, 1.7, 7.8)	125.9
1’	-	130.3
1’-CO	-	169.0
2’, 6’	7.99 (2H, dd, 1.1, 7.2)	129.7 (2X)
3’, 5’	7.38 (2H, t, 7.2)	128.2 (2X)
4’	7.51 (1H, tt, 1.1, 7.2)	132.9

Table 21 ^{1}H and ^{13}C-NMR spectral data of **A-6**. [(+) – zeylenol**, 1**]

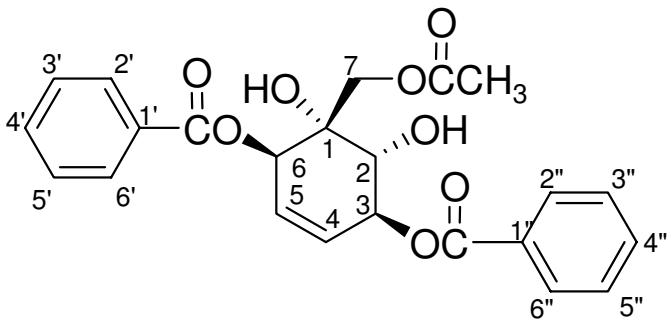
position	δ_H	δ_C
1	-	76.0
2	4.25 (1H, d, 6.2)	70.9
3	5.72 (1H, ddd, 1.7, 2.6, 6.2)	74.4
4	5.90 (1H, dd, 2.6, 10.1)	127.0
5	6.04 (1H, ddd, 1.7, 4.1, 10.1)	129.5
6	4.33 (1H, d, 4.1)	68.5
7	4.77 (1H, d, 12.3), 4.92 (1H, d, 12.3)	66.8
1', 1"	-	129.2 (2X)
2', 6'	8.01 (2H, dd, 1.2, 7.5)	129.8 (2X)
2", 6"	8.06 (2H, dd, 1.2, 7.5)	129.9 (2X)
3', 5' or 3", 5"	7.43 (2H, t, 7.5)	128.5 (2X)
3", 5" or 3', 5'	7.44 (2H, t, 7.5)	128.4 (2X)
4', 4"	7.59 (2H, dt, 1.2, 7.5)	133.5, 133.6
3-O<u>CO</u>Ar	-	167.2
7-O<u>CO</u>Ar	-	167.9



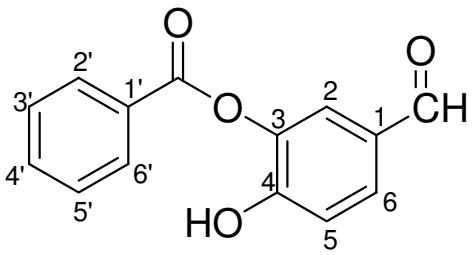
A-1



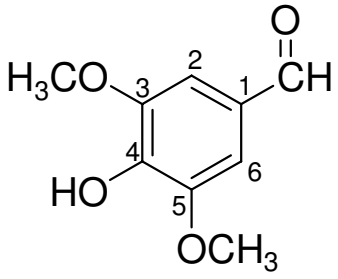
A-2



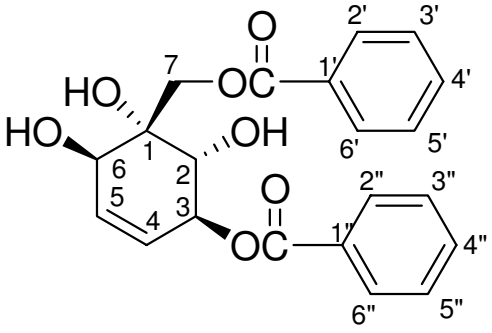
A-3



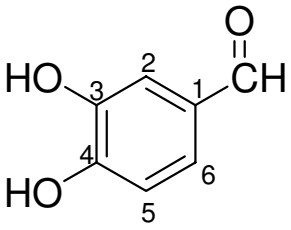
A-4


O
H3CO
2
3
1
CH
4
6
HO
5
OCH3

A-5


O
2'
3'
7
1'
HO
OC
4'
HO
1
OH
6'
5'
6
2
2"
3"
5
3
O
4
1"
OC
4"
6"
5"

A-6

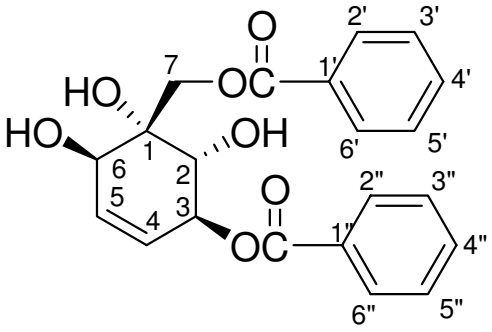

O
HO
2
3
1
CH
4
6
HO
5

A-7

RESULT AND DISCUSSION

The crude EtOAc extracts from the branches of *A. dulcis* were separated by chromatographic methods to yield two new polyoxygenated cyclohexene derivatives, (+)-anomianthol A (**2**) and (+)-anomianthol B (**3**) and a new aldehyde, 3-benzoyloxy-4-hydroxybenzaldehyde (**4**) together with four known compounds, (+)-zeylenol (**1**) [8], syringaldehyde (**5**), 3, 4-dihydroxybenzaldehyde (**6**) and benzoic acid (**7**). The structures were elucidated by spectroscopic analysis including 2D NMR techniques and by comparison of their spectral data with those previously reported in the literatures.

A-6



1 [(+)-zeylenol]

A-6 was isolated as colorless needles, m.p. 132-134 °C, which was shown to be optically active ($[\alpha]_D^{26}$ +121.9, *c* = 0.02, MeOH). Its IR spectrum showed absorption bands corresponding to the stretching of the hydroxyl group at 3451 cm^{-1}, the bands of the stretching of the carbonyl groups at 1694 and the C-O bond at 1284 and 1170 cm^{-1}. Its UV spectrum showed absorption peak corresponding to the aromatic ester moiety at λ_{max} 230 and 273 nm.

^{1}H-NMR spectrum (Table 21) of **A-6** contained ten aromatic protons of two monosubstituted aromatic rings appeared as two doublets of doublets of two hydrogens each at δ 8.01 (*J* = 1.2, 7.5 Hz, H-2', H-6') and δ 8.06 (*J* = 1.2, 7.5 Hz, H-2", H-6"), two triplets of two hydrogens each at δ 7.43 (*J* = 7.5 Hz, H-3', H-5' or H-3", H-5") and δ 7.44 (*J* = 7.5 Hz, H-3", H-5" or H-3', H-5'), and a doublet of triplets of two hydrogens at δ 7.59 (*J* = 1.2, 7.5 Hz, H-4', H-4"). Three oxymethine protons were indicated by two doublets of one hydrogen each at δ 4.25 (*J* = 6.2 Hz, H-2) and δ 4.33 (*J* = 4.1 Hz, H-6), and a doublet of doublet of

doublets of one hydrogen at δ 5.72 (*J* = 1.7, 2.6, 6.2 Hz, H-3). Two olefinic protons appeared as a doublet of doublets at δ 5.90 (*J* = 2.6, 10.1 Hz, H-4) and a doublet of doublet of doublets at δ 6.04 (*J* = 1.7, 4.1, 10.1 Hz, H-5). Two doublets at δ 4.77 (*J* = 12.3 Hz) and δ 4.92 (*J* = 12.3 Hz) were assigned to two methylene protons (H_a-7 and H_b-7).

The ^{13}C-NMR spectral data of **A-6** (Table 21) contained twelve aromatic carbons at δ 133.5 and 133.6 (C-4', C-4"), 129.2 (C-1', C-1"), 129.8 (C-2', C-6'), 129.9 (C-2", C-6"), 128.5 (C-3', C-5' or C-3", C-5") and 128.4 (C-3", C-5" or C-3', C-5') were assigned to two aromatic rings which was corresponded with the ^{1}H-NMR spectral data. Two ester carbonyl carbons appeared at δ 167.2 and 167.9. Three oxymethine carbons appears at δ 70.9 (C-2), 74.4 (C-3) and 68.5 (C-6). The ^{13}C-NMR spectral data of **A-6** also contained two olefinic carbons at δ 127.0 (C-4) and 129.5 (C-5), an oxyquaternary carbon at δ 76.0 (C-1), and a methylene carbon at δ 66.8 (C-7). The ^{13}C-NMR spectral data of **A-6** were assigned by a combination of DEPT, 2D HMQC and 2D HMBC experiments.

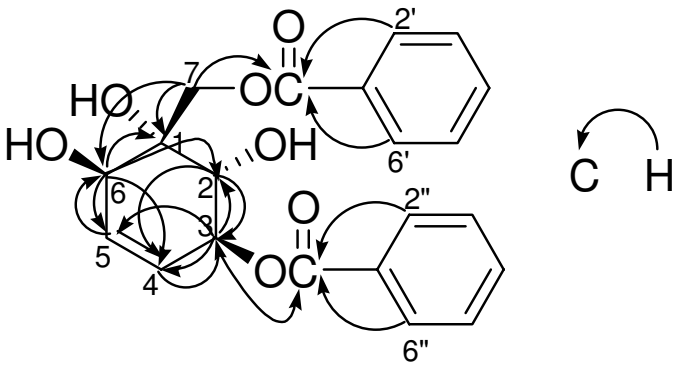
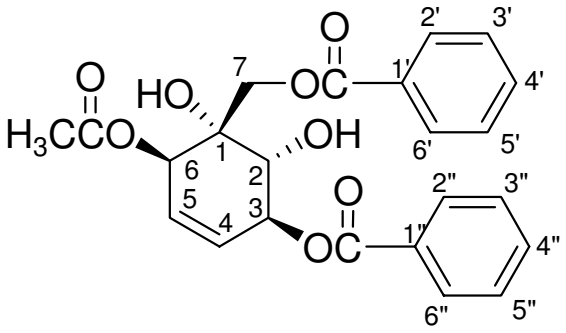


Figure 2 Selected 2D HMBC correlations of **A-6**.

The two benzoyloxy groups attached to C-7 and C-3 were established by the 2D HMBC correlations (Fig. 2). The carbonyl carbon of benzoyloxy group at δ 167.9 (7-O<u>CO</u>Ar) had long-range correlations to the aromatic protons at δ 8.01 (H-2' and H-6') and the methylene protons (H_a-7 and H_b-7) at δ 4.77 and 4.92, while the carbonyl group at δ 167.2 (3-O<u>CO</u>Ar) had correlations to the aromatic protons at δ 8.06 (H-2" and H-6") and oxymethine proton at δ 5.72 (H-3) implying that the two benzoyloxy groups connected to C-7 and C-3, respectively.

The HMBC correlations on cyclohexene ring were observed between oxymethine carbon (C-6) at δ 68.5 and olefinic proton (H-5) at δ 6.04 and methylene protons (H_{ab}-7) at δ 4.77 and 4.92, and between quaternary carbon (C-1) at δ 76.0 and H-6 at δ 4.33. In addition, the oxymethine carbon (C-2) at δ 70.9 had correlations to H-3 (δ 5.72) and H-6 (δ 4.33), and C-3 at δ 74.4 had correlations to H-2 (δ 4.25) and H-4 (δ 5.90). The 2D HMBC spectrum of **A-6** also showed correlations between olefinic carbons C-4 (δ 127.0) and H-6 (δ 4.33) and between C-5 (δ 129.5) and H-3 (δ 5.72). On the basis of the above evidences and by comparison the spectral data with those previous, reported [8], **A-6** was characterized as (+) – zeylenol (**1**).

A-2



2 [(+)-anomianthol A]

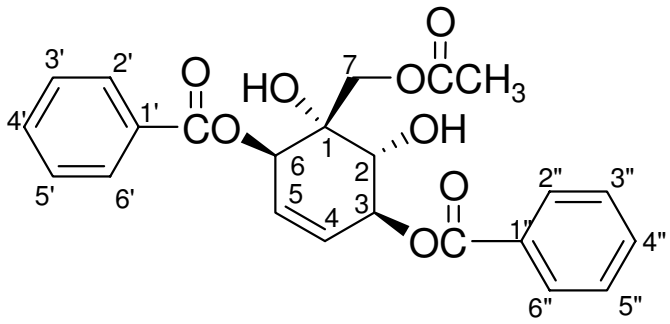
A-2 was isolated as a colorless solid, m.p. 119-120 °C, which was shown to be optically active ($[\alpha]_D^{26}$ +100.0, *c* = 0.02, MeOH). Its IR spectrum showed absorption bands corresponding to the stretching of the hydroxyl group at 3450 cm^{-1}, the ester carbonyl at 1693 cm^{-1} and the C-O bond at 1299 and 1171 cm^{-1}. Its UV spectrum showed the absorption peaks corresponding to the aromatic ester moieties at λ_{max} 230, 273 and 280 nm.

^{1}H-NMR spectrum (Table 18) of **A-2** was very similar to that of **A-6** [(+)-zeylenol)]. The spectrum contained ten aromatic protons of two monosubstituted aromatic rings appeared as two doublets of doublets of two protons each at δ 7.95 (*J* = 1.3, 7.8 Hz, H-2', H-6' or H-2", H-6") and δ 8.03 (*J* = 1.3, 7.8 Hz, H-2", H-6" or H-2', H-6'), a doublet of triplets of four protons at δ 7.40 (*J* = 1.3, 7.8 Hz, H-3', H-5', H-3", H-5") and a doublet of triplets of two protons at δ 7.55 (*J* = 1.3, 7.8 Hz, H-4', H-4"). Three oxymethine protons were indicated by two doublets of one hydrogen each at δ 4.26 (*J* = 6.5 Hz, H-2) and δ 5.56 (*J* = 1.7 Hz, H-6), and a

doublet of doublets of one hydrogen at δ 5.78 (*J* = 1.7, 6.5 Hz, H-3). Two olefinic protons appeared as a doublet at δ 5.93 (*J* = 1.7 Hz, H-4, H-5). Two methylene protons (H_a-7 and H_b-7) were observed as two doublets at δ 4.58 (*J* = 12.0 Hz) and δ 4.82 (*J* = 12.0 Hz). In addition, a singlet at δ 2.10 was assigned to an acetyl group.

The ^{13}C-NMR spectral data of **A-2** (Table 19) contained twenty-three carbon atoms. Twelve aromatic carbons at δ 133.3 and 133.4 (C-4', C-4"), 129.4 (C-1', C-1"), 129.6 (C-2', C-6' or C-2", C-6"), 129.8 (C-2", C-6" or C-2', C-6'), and 128.4 (C-3', C-5', C-3", C-5") were assigned to two aromatic rings which was corresponded with the ^{1}H-NMR spectral data. Two aromatic carbonyl carbons appeared at δ 167.2 (7-O<u>CO</u>Ar and 3-O<u>CO</u>Ar) and one acetoxy carbonyl carbon appeared at δ 170.2 (6-O<u>CO</u>CH_3). Three oxymethine carbons appears at δ 71.1 (C-2), 73.4 (C-3) and 70.6 (C-6). The ^{13}C-NMR spectral data of **A-2** also contained two olefinic carbons at δ 128.6 (C-4) and 126.6 (C-5), an oxyquaternary carbon at δ 74.5 (C-1), and a methylene carbon at δ 66.4 (C-7). In addition, an acetoxy methyl carbon at δ 20.9 was also observed. The ^{13}C-NMR spectral data of **A-2** were assigned by a combination of DEPT, 2D HMQC and 2D HMBC experiments.

A-3



3 [(+)-anomianthol B]

A-3 was isolated as a pale yellow resin, which was shown to be optically active ($[\alpha]_D^{26}$ +76.1, *c* = 0.02, MeOH). Its IR spectrum showed absorption bands corresponding to the stretching of the hydroxyl group at 3450 cm^{-1}, the carbonyl groups at 1715 cm^{-1} and the C-O bond at 1269 and 1170 cm^{-1}. Its UV spectrum showed absorption peak corresponding to the aromatic ester moieties at λ_{max} 230, 274 and 281 nm.

The NMR spectra (^1H and ^{13}C) (Tables 18 and 19) of **A-3** were very similar to those of **A-2** except olefinic protons (H-4 and H-5). ^1H-NMR spectrum (Table 18) of **A-3**, an olefinic proton (H-4) appeared as a doublet of doublets at δ 6.01 (*J* = 2.5, 10.2 Hz) and an olefinic proton (H-5) appeared as a doublet of doublet of doublets at δ 6.08 (*J* = 1.4, 3.6, 10.2 Hz) whereas the olefinic protons (H-4 and H-5) of **A-2** showed at δ 5.93 (2H) as a doublet (*J* = 1.7 Hz).

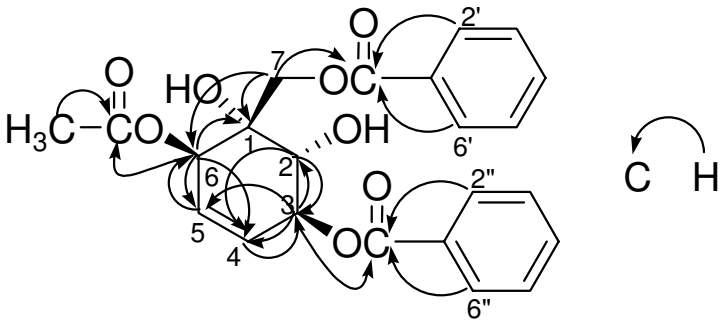


Figure 3 Selected 2D HMBC correlations of **A-2**.

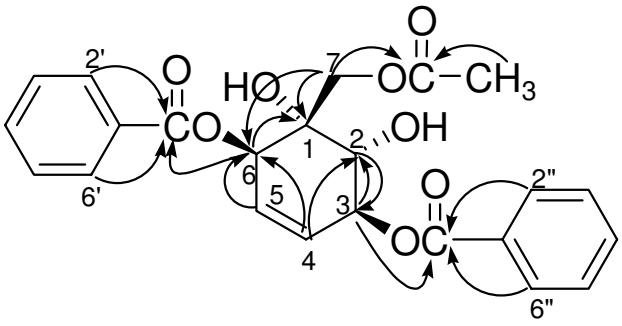
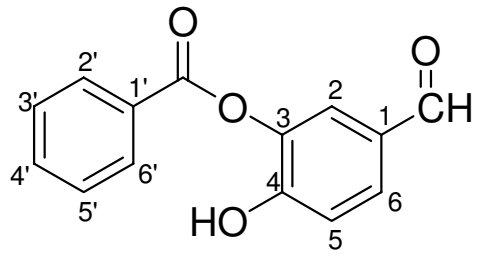


Figure 4 Selected 2D HMBC correlations of **A-3**.

The HMBC correlations of **A-2** and **A-3** were similar to those of (+)-zeylenol (**1**) (Fig. 2). The HMBC correlations of **A-2**, methylene protons (δ 4.58 and δ 4.82) and H-3 (δ 5.78) had correlations to the carbonyl carbons of benzoyl groups (δ 167.0). This suggested that two benzoyloxy groups connected to C-7 and C-3. Moreover, H-6 (δ 5.56) had correlations to the acetoxy carbonyl carbon (δ 170.2) implying that the acetyl group was attached to C-6. The HMBC correlations of **A-3**, correlations between methylene protons at δ 4.43 (H_a-7) and 4.64 (H_b-7) and acetoxy carbonyl carbon (δ 171.6) and between H-6 (δ 5.70) and H-3 (δ 5.79) and carbonyl carbons of the two benzoyloxy groups at δ 165.7 and 166.9, respectively, were observed. This suggested that the acetoxy carbonyl

connected to C-7 and the two benzoyloxy groups connected to C-6 and C-3. On the basis of the above evidences, **A-2** and **A-3** were characterized as (+)-anomianthol A (**2**) and (+)- anomianthol B (**3**), respectively.

A-4



4 (3-benzoyloxy-4-hydroxybenzaldehyde)

A-4 was isolated as colorless needles, m.p. 88-92 °C. Its IR spectrum showed absorption bands corresponding to the stretching of the C-H of the aldehyde group at 2726 cm^{-1}, the carbonyl groups at 1697 cm^{-1} and the C-O bond at 1288 and 1169 cm^{-1}. Its UV spectrum also showed absorption band corresponding to the aromatic ester at λ_{max} 227 and 278 nm.

^{1}H-NMR spectrum (Table 20) of **A-4** contained eight aromatic protons separated into two groups. Protons of a monosubstituted aromatic ring appeared as a doublet of doublets of two protons at δ 7.99 (J = 1.1, 7.2 Hz, H-2', H-6'), a triplet of two protons at δ 7.38 (J = 7.2 Hz, H-3', H-5'), and a triplet of triplets of one proton at δ 7.51 (J = 1.1, 7.2 Hz, H-4'). Protons of a 1, 3, 4-trisubstituted aromatic ring appeared as two doublets of one proton each at δ 7.27 (J = 1.7 Hz, H-2) and δ 6.89 (J = 7.8 Hz, H-5), and a doublet of doublets of one proton at δ 7.25 (J = 1.7, 7.8 Hz, H-6). In addition, an aldehyde proton at δ 9.66 appeared as a singlet was also observed.

The ^{13}C-NMR spectral data of **A-4** (Table 20) contained fourteen carbons. Six aromatic carbons at δ 130.3 (C-1'), 129.7 (C-2', C-6'), 128.2 (C-3', C-5') and 132.9 (C-4') were assigned to the monosubstituted aromatic rings which was in good agreement with the ^{1}H-NMR spectral data. Six aromatic carbons of the trisubstitued aromatic ring appeared at δ 129.3 (C-1), 113.9 (C-2), 145.2 (C-3), 152.0 (C-4), 114.9 (C-5) and 125.9 (C-6). In addition, an aldehyde carbon at δ 191.9 and a carbonyl carbon at δ 169.0 were also observed. The ^{13}C-NMR spectral data of **A-4** were assigned by a combination of DEPT, 2D HMQC and 2D HMBC experiments.

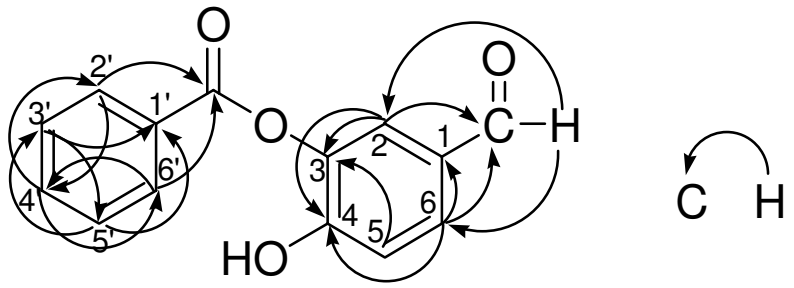
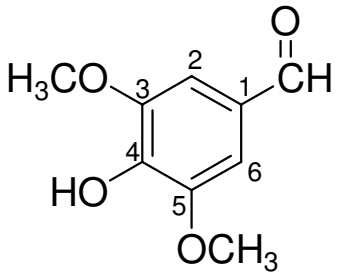


Figure 5 Selected 2D HMBC correlations of **A-4**.

The HMBC correlations of **A-4** (Fig. 5) were observed between carbonyl carbon (1'-CO) at δ 169.0 and aromatic protons (H-2' and H-6') at δ 7.99 and between aldehyde carbon at δ 191.9 and H-2 at δ 7.27 and H-6 at δ 7.25. In addition, H-2 at δ 7.27 had correlations to C-3 (δ 145.2) and C-4 (δ 152.0) and H-6 at δ 7.25 had correlations to C-1 (δ 129.3) and C-4 (δ 152.0) and the aldehyde carbon (δ 191.9). In the HMBC spectrum, correlations between the aldehyde proton (δ 9.66) and C-2 (δ 113.9) and C-6 (δ 125.9) were also observed. On the basis of the above evidences, 3-benzoyloxy-4-hydroxybenzaldehyde (**4**) was assigned for **A-4**.

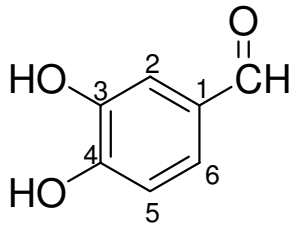
A-5



5 (syringaldehyde)

A-5 was isolated as a pale brown solid, m.p. 110.0-113.0 °C. ^{1}H-NMR spectrum (Table 17) of **A-5** contained two aromatic protons appeared as a singlet at δ 7.18 (H-2 and H-6). In addition, a singlet of six protons of two methoxyl groups at δ 4.00 (6H) and a singlet of an aldehyde proton at δ 9.84 (1H) were also observed. **A-5** showed identical ^{1}H-NMR spectral data with those of syringaldehyde reported in the literature [9] together with its melting point at 110.0 – 113.0 °C (lit.[10] 113 °C). Syringaldehyde (**5**) was therefore assigned for **A-5**.

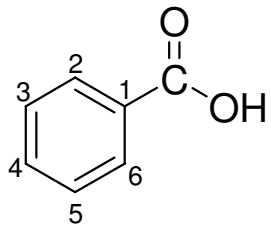
A-7



6 (3, 4-dihydroxybenzaldehyde)

A-7 was isolated as a pale brown solid, m.p. 150.0-155.0 °C. ^{1}H-NMR spectrum (Table 17) of **A-7** contained three aromatic protons appeared as two doublet at δ 7.28 (J = 1.7 Hz, H-2) and δ 6.90 (J = 7.8 Hz, H-5), and a doublet of doublets at δ 7.25 (J = 1.7, 7.8 Hz, H-6). In addition, a singlet at δ 9.65 (1H) were observed and assigned as an aldehyde proton. ^{1}H-NMR spectrum of **A-7** was identical with those of 3, 4-dihydroxybenzaldehyde reported in the literature [11]. **A-7** was thus identified as 3, 4-dihydroxybenzaldehyde (**6**).

A-1



7 (benzoic acid)

A-1 was isolated as a white solid, m.p. 121.0-123.5 °C. ^{1}H-NMR spectrum (Table 16) of **A-1** contained five aromatic protons appeared as a doublet of doublets at δ 7.99 (J = 1.1, 7.2 Hz, H-2, H-6), a triplet at δ 7.28 (J = 7.2 Hz, H-3, H-5), and a triplet of triplets at δ 7.45 (J = 1.1, 7.2 Hz, H-4). ^{1}H-NMR spectrum of **A-1** was identical with those of benzoic acid reported in the literature [12]. Together with its melting point at 121.0 -123.5 °C (lit.[13] 122.4 °C), **A-1** was identified as benzoic acid (**7**).

REFERENCES

1. A. Sinz, R. Matusch, L. Witte, T. Santisuk, S. Chaichana, V. Reutrakul. *Biochem. Syst. Ecol*., **26**, 139 (1998).
2. A. Sinz, R. Matusch, T. Santisuk, S. Chaichana, V. Reutrakul. *Biochem. Syst. Ecol*., **26**, 361 (1998).
3. A. Sinz, R. Matusch, F. V. Elsacker, T. Santisuk, S. Chaichana, V. Reutrakul. *Phytochemistry*, **50**, 1069 (1999).
4. P. Tuntiwachwuttikul, O. Pancharoen, W. A. Bubb, T. W. Hambley, W. C. Taylor, V. Reutrakul. *Aust. J. Chem.*, **40**, 2048 (1987).
5. N. Fukuda, M. Yonemitsu, T. Kimura. *Chem. Pharm. Bull.*, **13**, 156 (1983).
6. A. U. Rahman, M. K. Ahatti, F. Akhtar, M. I. Choudhhary. *Phytochemistry*, **31**, 2869 (1992).
7. C. Kamperdick, N. H. Van, T.V. Sung. *Phytochemistry*, **61**, 991 (2002).
8. Y. Takeuchi, Q. Cheng, Q. W. Shi, T. Sugiyama. T. Olitani. *Biosci. Biotechnol. Biochem.*, **65** (6), 1395-1398 (2001).
9. The Aldrich Library of NMR Spectra Ed II, **2**, p.124C (1983).
10. Merck Index, Ed XII, p.1543 (1996).
11. The Aldrich Library of NMR Spectra Ed II, **2**, p.119A (1983).
12. The Aldrich Library of NMR Spectra Ed II, **2**, p.182C (1983).
13. Merck Index, Ed XII, p.183 (1996).

CHAPTER 2

CHEMICAL CONSTITUENTS OF THE BRANCHES OF *DALBERGIA COCHINCHINENSIS* PIERRE

INTRODUCTION

Dalbergia is a large genus of small to medium-size trees and shrubs in the pea family, Fabaceae or Leguminosae, subfamily Faboideae. The genus, with between 150-300 species, has a wide distribution, native to the tropical regions of Central and South America, Africa, Madagascar, and Southern Asia. [1]

Dalbergia cochinchinensis Pierre is perennial non-climbing tree grows widely in lowland and submontane broadleaved, dense evergreen tropical forests or semi-deciduous forest up to 1000 m altitude. The plant is a native of Thailand and Vietnam and an occurrence reported in Cambodia and Laos. Its common name is *cam lai* (Vietnam), *phayuung* (Thailand), *nhoung* (Cambodia) and *khanhoung* (Lao). Its vernacular names in Thailand are *kra-yung* (Khmer-Surin), *daeng cheen* (Prachin Buri), *praduu tom* (Chanthaburi), *praduu laai* (Chon Buri), *praduu sen* (Trat), *phayuung mai* (Sayaburi). Its English trade names are *Thailand rosewood*, *Siamese rosewood*. [2, 3]

D. cochinchinensis is a medium to large evergreen tree, 8-30 m in height and 60 cm in trunk diameter. The bark is brownish-yellow, longitudinally fissured, sometimes peeling into fragments. The crown is spherical, and leaves are pinnately compound, alternate, 15–20 cm long. Leaflets number 7–9, are oval, alternate or sub-opposite, top obtuse, or shortly acuminate, base cuneate, 3–5 cm long and 1.8–2.5 cm wide, and leathery. The terminal leaflet is the largest. Veins are slightly prominent. Inflorescence is paniculate, axillary, bracteate, and bracteolate.

Figure 6 *Dalbergia cochinchinensis* Pierre.

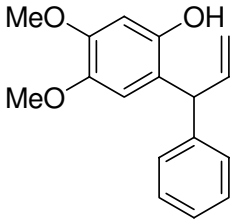
This species has white flowers. Flowers are sepals connate with 5-dented at the top and glabrous and standard rectangular petals with straight claws and 9 stamens. Fruits are 5–6 cm long, 1 cm wide, and tapering, with very flat indehiscent pods, 5–6 cm long and 1 cm wide. Generally, a pod contains one or two seeds. It flowers between May and July and seed matures in September-November. The seed is mature when the pod changes color to dark brown. Seed collection can be done by climbing the mother tree and cutting small branches or allowing the seed to drop onto tarpaulins on the ground. To minimize insect predation the seed can be collected as soon as the color turns from green to yellow.

D. cochinchinensis is categorized as a “luxury" timber in Cambodia, whereas in Vietnam it is considered a “first class prime timber”, as it is hard, durable, easy to work and resistant to insects and termites. The distinctive sapwood and heartwood makes beautiful patterns when sawn. Sapwood is grayish, whereas heartwood is brown-red or black, with a fine texture. It is very hard and heavy with a density of 1.09. It is very popular in the manufacture of luxury furniture (beds, wardrobes, desks etc.) and in wood turnery, fine-art, musical instruments, sewing-machines and sports equipment. It is also used as a decorative timber, for example, in passenger ships and for instrument cases. Because of its strength and durability it is suitable for all kinds of construction work, for doors, window frames and wagon building. It is also used for heavy-duty striking tools such as hammers, felling axes and agricultural implements such as ploughs, harrows, rollers, etc. In cart and carriage building, it is used for felloes, spokes, poles, shafts, rims, etc.

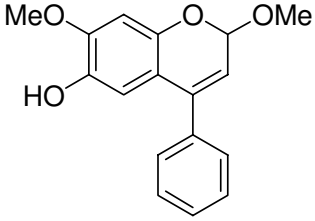
In 1968, Donnelly *et al*. [4] found a new compound, (*R*)-5-*O*-methyllatifolin from the heartwood of *D. cochinchinensis* together with four known compounds, (*R*)-latifolin, (*R*)-4-methoxydalbergione, benzoic acid and salicylic acid.

In 1996, Satake *et al*. [5] reported twelve phenolic compounds having antiandrogenic activity.

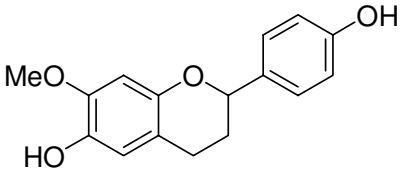
In 1997, Satake *et al*. [6] isolated four new compounds, 9-hydroxy-6,7-dimethoxydalbergiquinol (**29**), 6-hydroxy-2,7-dimethoxyneoflavene (**30**), 6,4'-dihydroxy-7-methoxyflavan (**31**) and 2,2',5-trihydroxy-4-methoxybenzophenone (**32**), from the stems of *D. cochinchinensis*. , together with 7-hydroxy-6-methoxyflavone (**33**) which was isolated for the first time from this plant, and eight known phenolic compounds, latifolin, 2,5-dihydroxy-4-methoxybenzophenone, 5-*O*-methyllatifolin, methoxydalbergion, 6,4'-dihydroxy-7-methoxyflavanone, liquiritigenin, calycosin and isoliquiritigenin. The structures were elucidated by spectroscopic analysis and comparison of their spectral data with those reported previously. Of these newly isolated compounds 9-hydroxy-6,7-dimethoxydalbergiquinol and 6-hydroxy-2,7-dimethoxyneoflavene showed potent inhibitory activity towards 5α-dihydrotestosterone (DHT) which binds with an androgen receptor to form a DHT-receptor complex that causes androgen-dependent diseases.



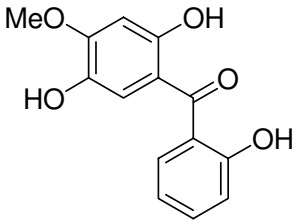
9-hydroxy-6, 7-dimethoxydalbergiquinol (**29**)



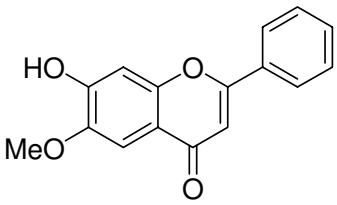
6-hydroxy-2,7-dimethoxyneoflavene (**30**)



6,4'-dihydroxy-7-methoxyflavan (**31**)

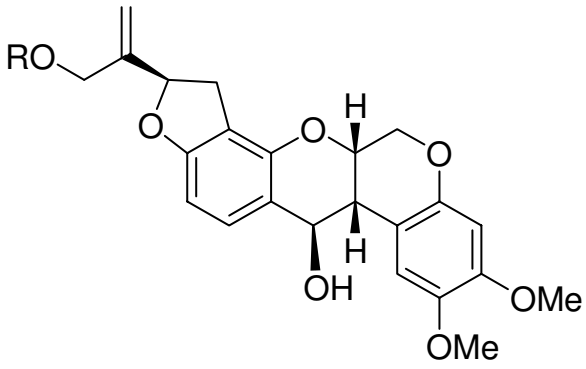


2,2',5-trihydroxy-4-methoxybenzophenone (**32**)



7-hydroxy-6-methoxyflavone (**33**)

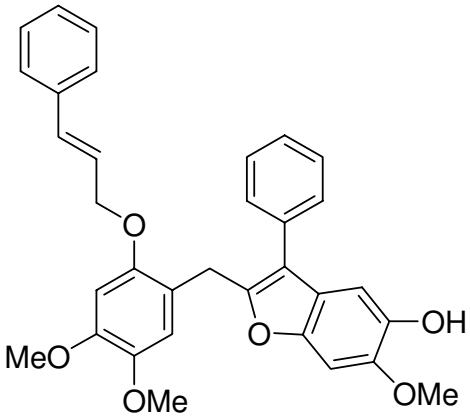
In 1999, Svasti *et al*. [7] reported the purification and structural characterization of a novel isoflavonoid β-glucoside: dalcochinin-8′-O-β-D-glucoside (**34**) from Thai Rosewood seeds. This compound was shown to be a β-glucoside by enzymatic hydrolysis with purified *D. cochinchinensis* β-glucosidase to give the aglycone **35** and D-glucose (analyzed by TLC and HPLC). The structures were also elucidated by spectroscopic analysis.



34: R = β-D-glucose

35: R = H

In 2003, Hayashi *et al*. [8] isolated three new phenolic compounds (**36-38**), along with five known phenolics, 4'-hydroxy-2'-methoxychalcone (**39**), latinone (**40**), dalbergiphenol (**41**), 7-hydroxyflavanone, and dalbergin, from the stems of *D. cochinchinensis*. The structures were determined on the basis of spectroscopic analysis and comparison with the literature data. These compounds were isolated for the first time from this plant. The inhibitory activity against testosterone 5-reductase, which causes androgen-dependent diseases, was also examined for the selected compounds.



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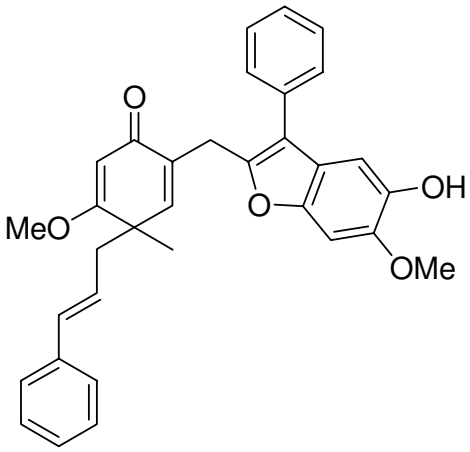

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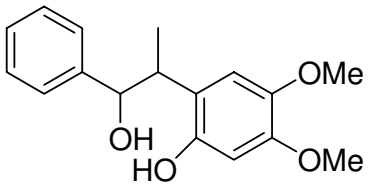

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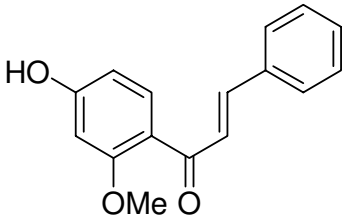
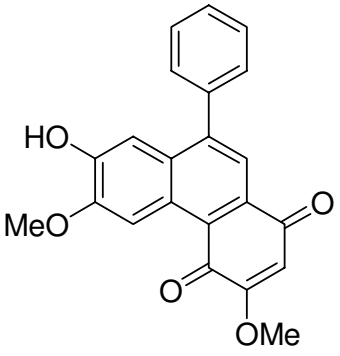
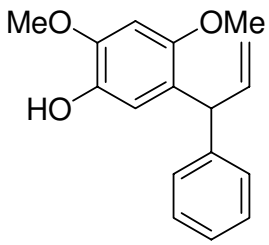


4'-hydroxy-2'-methoxychalcone (**39**)



latinone (**40**)



dalbergiphenol (**41**)

EXPERIMENTAL

Optical rotations were measured in methanol solution with sodium D line (590 nm) on a JASCO P-1010 polarimeter. Ultraviolet spectra (UV) were measured with a Shimadzu UV-240 spectrophotometer. Infrared spectra (IR) were recorded with a JASCO A-302 spectrophotometer. Major bands (ν_{max}) were recorded in wavenumber (cm^{-1}). ^{1}H-and ^{13}C-NMR were measured in $CDCl_3$ or $CDCl_3$-CD_3OD on a Bruker AVANCE 300 (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR) spectrometer. Chemical shifts are in δ (ppm) with tetramethylsilane as an internal standard. Coupling constants (*J*) are given in Hz. The signals in the ^{1}H- and ^{13}C-NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC and HMBC. MS were recorded on a VG 7070 mass spectrometer operating at 70 eV or with a VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectra. HRMS were recorded on a Bruker MicrOTOF mass spectrometer. Column chromatography was carried out using Kieselgel 60 (Merck, 0.063-0.200 mm or 0.015-0.040 mm) and Lichroprep RP-18 (Merck, 40-63 μm). Pre-coated silica gel 60 F_{254} (Merck, layer thickness 0.25 mm) and pre-coated RP-18 F_{254s} (Merck) were used for thin-layer chromatography (TLC) and the compounds were visualized under ultraviolet light or by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. Preparative layer chromatography (PLC) was performed on pre-coated silica gel 60 F_{254} (Merck, 20x20 cm, layer thickness 0.25, 0.5 or 1.0 mm). All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

Plant material

The branches of *Dalbergia cochinchinensis* Pierre (Fabaceae or Leguminosae) were collected from Kaeng Tana National Park, Ubonratchathanee, Thailand, in September 2004. A voucher specimen (SS128/58) has been deposited at the National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Paholyothin Road, Klong 1, Klong Luang, Pathomthani 12120, Thailand.

Extraction and isolation of the branches of *D. cochinchinensis*

The air dried branches of *D. cochinchinensis* (3.21 kg) were extracted with 95% EtOH (3x30.0 l) at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure to give a dark brown oil (85.6 g, DC-1).

DC-1 (a dark brown oil, 85.6 g) was suspended in water (400 ml) and extracted first with EtOAc 3 times (500 ml) and then with *n*-BuOH 3 times (300 ml) in a separatory funnel. The extracts were evaporated under reduced pressure to give a brown wax of the EtOAc-soluble extract (21.8 g, DC-1E), a dark brown wax of the *n*-BuOH-soluble extract (16.3 g, DC-1B).

DC-1E (21.8 g) was separated by flash column chromatography using silica gel 60 [Merck, 0.015-0.040 mm, diameter x height (13.3 cm x 5.0 cm)]. The column was eluted with 1000 ml (2 x 500 ml) each of hexane, gradient of EtOAc/hexane, and gradient of MeOH/EtOAc. The fractions obtained were evaporated under reduced pressure to give 28 fractions (Table 22).

Table 22 Fractions obtained from DC-1E.

Fraction No.	Eluent	Weight (g)	Physical characteristic
DC-1E-1	10 % EtOAc/ hexane	0.4725	a pale yellow oil
DC-1E-2	15 % EtOAc/ hexane	0.5765	a pale yellow oil
DC-1E-3	20 % EtOAc/ hexane	0.7666	a green wax
DC-1E-4	25 % EtOAc/ hexane	0.6152	a dark green wax
DC-1E-5	30 % EtOAc/ hexane	0.2667	a dark green wax
*DC-1E-6	35 % EtOAc/ hexane	0.3555	a dark green wax
*DC-1E-7	40 % EtOAc/ hexane	0.2942	a dark green wax
*DC-1E-8	45 % EtOAc/ hexane	0.3572	a green wax
*DC-1E-9	50 % EtOAc/ hexane	0.3889	a green wax
*DC-1E-10	55 % EtOAc/ hexane	0.4223	a green wax
*DC-1E-11	60 % EtOAc/ hexane	0.5839	a green wax
*DC-1E-12	65 % EtOAc/ hexane	0.5920	a green wax
DC-1E-13	70 % EtOAc/ hexane	0.2931	a dark brown wax

* Fractions were further investigated.

Table 22 Fractions obtained from DC-1E (continued).

Fraction No.	Eluent	Weight (g)	Physical characteristic
DC-1E-14	75 % EtOAc/ hexane	0.2782	a dark brown wax
DC-1E-15	80 % EtOAc/ hexane	0.2519	a dark brown wax
DC-1E-16	85 % EtOAc/ hexane	0.2691	a dark brown wax
DC-1E-17	90 % EtOAc/ hexane	0.7450	a dark brown wax
DC-1E-18	95 % EtOAc/ hexane	0.3972	a dark brown wax
DC-1E-19	EtOAc	0.7400	a dark brown wax
DC-1E-20	1 % MeOH/ EtOAc	0.6495	a dark brown wax
DC-1E-21	3 % MeOH/ EtOAc	0.7328	a dark brown wax
DC-1E-22	5 % MeOH/ EtOAc	0.8516	a dark brown wax
DC-1E-23	8 % MeOH/ EtOAc	0.8224	a dark brown wax
DC-1E-24	10 % MeOH/ EtOAc	0.8823	a dark brown wax
DC-1E-25	25 % MeOH/ EtOAc	2.2761	a dark brown wax
DC-1E-26	50 % MeOH/ EtOAc	6.5232	a dark brown wax
DC-1E-27	75 % MeOH/ EtOAc	7.3299	a dark brown wax
DC-1E-28	MeOH	0.4230	a dark brown wax

* Fractions were further investigated.

Fraction DC-1E-6

Fraction DC-1E-6 (a dark green wax, 355.5 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 30 g) using CH_2Cl_2/MeOH/H_2O (800:3:1, 700:3:1, 500:3:1, 400:3:1, 300:3:1, 200:3:1, 100:3:1, 80:3:1, 50:3:1) as the eluent. The fractions obtained were combined on the basis of their behaviors on TLC and evaporated under reduced pressure to give 12 fractions. (Table 23)

Table 23 Fractions obtained from DC-1E-6.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-6-1	11.4	an orange oil
DC-1E-6-2	6.4	a bright yellow oil
DC-1E-6-3	51.8	a pale brown oil
DC-1E-6-4	36.6	a yellow semisolid
*DC-1E-6-5	50.9	a yellow oil
*DC-1E-6-6	43.9	a green solid
DC-1E-6-7	14.9	a yellow oil
DC-1E-6-8	15.8	a yellow oil
DC-1E-6-9	16.9	a yellow oil
DC-1E-6-10	18.7	a yellow oil + a colorless solid
DC-1E-6-11	13.3	a yellow oil
DC-1E-6-12	22.4	a pale yellow solid

* Fractions were further investigated.

Fraction DC-1E-6-5 (a yellow oil, 50.9 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 10 g) using CH_2Cl_2/MeOH/H_2O (800:3:1, 500:3:1, 100:3:1, 50:3:1) as the eluent to give 5 fractions. (Table 24)

Table 24 Fractions obtained from DC-1E-6-5.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-6-5-1	1.6	a yellow oil
DC-1E-6-5-2	3.7	a yellow oil
DC-1E-6-5-3	27.0	a pale yellow solid
DC-1E-6-5-4	6.4	a pale yellow solid
DC-1E-6-5-5	6.5	a yellow oil

DC-1E-6-5-3 (27.0 mg) was identified as compound **D-1**.

Fraction DC-1E-6-6 (a green solid, 43.9 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 2 plates) using CH_2Cl_2/ MeOH/H_2O (80:3:1; 3 runs) as the developing solvent to give 6 fractions. (Table 25)

Table 25 Fractions obtained from DC-1E-6-6.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-6-6-1	2.8	a green oil
DC-1E-6-6-2	2.7	a yellow oil
DC-1E-6-6-3	2.4	a pale yellow solid
DC-1E-6-6-4	7.3	a pale yellow solid
DC-1E-6-6-5	2.5	a pale yellow solid
DC-1E-6-6-6	3.3	a white solid

DC-1E-6-6-4 (7.3 mg) was identified as compound **D-2** (partially pure).

Fraction DC-1E-7

Fraction DC-1E-7 (a dark green wax, 368.7 mg) was crystallized from MeOH to give DC-1E-7-ppt (a pale brown solid, 74.5 mg). The filtrate was evaporated to give DC-1E-7-f (a dark brown wax, 294.2 mg)

Fraction DC-1E-7-ppt (a pale brown solid, 74.5 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 1.00 mm, 2 plates) using CH_2Cl_2/MeOH/H_2O (80:3:1; 2 runs) as the developing solvent to give 2 fractions (Table 26)

Table 26 Fractions obtained from DC-1E-7-ppt.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-7-ppt-1	5.7	a cream solid
DC-1E-7-ppt-2	1.6	a pale yellow solid

DC-1E-7-ppt-1 (5.7 mg) was identified as compound **D-2**

Fraction DC-1E-7-f (a dark brown wax, 294.2 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 30 g) using CH_2Cl_2/MeOH/H_2O (800:3:1, 400:3:1, 300:3:1, 200:3:1, 100:3:1, 50:3:1, 30:3:1, 20:3:1) as the eluent to give 11 fractions. (Table 27)

Table 27 Fractions obtained from DC-1E-7-f.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-7-f-1	5.7	a yellow solid
DC-1E-7-f-2	30.9	a yellow oil
DC-1E-7-f-3	7.9	a yellow oil
DC-1E-7-f-4	13.3	a pale yellow solid
DC-1E-7-f-5	9.4	a pale yellow solid
DC-1E-7-f-6	4.4	a brown solid
DC-1E-7-f-7	3.9	a yellow oil + a white solid
DC-1E-7-f-8	5.1	a brown oil
DC-1E-7-f-9	15.2	a yellow oil
DC-1E-7-f-10	10.8	a yellow oil
DC-1E-7-f-11	5.5	a orange oil

DC-1E-7-f-4 (13.3 mg) was crystallized with MeOH/$CHCl_3$ to give colorless needles and identified as compound **D-2**.

Fraction DC-1E-8

Fraction DC-1E-8 (a dark brown wax, 357.2 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 35 g) using CH_2Cl_2/MeOH/H_2O (1000:3:1, 800:3:1, 400:3:1, 300:3:1, 200:3:1, 100:3:1, 80:3:1, 50:3:1) as the eluent to give 14 fractions. (Table 28)

Table 28 Fractions obtained from DC-1E-8.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-8-1	12.0	a bright yellow solid
DC-1E-8-2	22.5	a brown semisolid
DC-1E-8-3	25.6	a yellow oil
DC-1E-8-4	16.6	a yellow oil
DC-1E-8-5	17.1	a yellow wax
DC-1E-8-6	7.2	a yellow oil
*DC-1E-8-7	26.9	a brown solid
DC-1E-8-8	32.4	a brown wax
DC-1E-8-9	25.5	a yellow oil
DC-1E-8-10	14.8	a yellow oil
DC-1E-8-11	23.2	a brown wax
*DC-1E-8-12	25.6	a brown oil + a white solid
DC-1E-8-13	18.4	a brown solid
*DC-1E-8-14	25.4	a brown solid

* Fractions were further investigated.

Fraction DC-1E-8-7 (a brown solid, 26.9 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 1.00 mm, 1 plate) using benzene/EtOAc (5:1; 4 runs) as the developing solvent to give 2 fractions. (Table 29)

Table 29 Fractions obtained from DC-1E-8-7.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-8-7-1	8.9	a pale yellow solid
DC-1E-8-7-2	11.8	a pale yellow solid

DC-1E-8-7-1 (8.9 mg) was identified as **D-2** (not pure)

Fraction DC-1E-8-12 (a brown oil + a white solid, 25.6 mg) was precipitated by $CHCl_3$ to give the precipitate (DC-1E-8-12-ppt, **D-3**) as a cream solid (6.7 mg) and the filtrate (DC-1E-8-12-f) as a brown wax (18.9 mg).

Acetylation of DC-1E-8-12-ppt (**D-3**)

A mixture of DC-1E-8-12-ppt (**D-3**, 6.7 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was heated at 80 °C for 2 hours. The mixture was allowed to stand at room temperature, water (10 ml) was added and the mixture was extracted with CH_2Cl_2 (3x10 ml). The CH_2Cl_2-soluble extract was then extracted with 10% aq.H_2SO_4 (3x10 ml) and washed with water (3x20 ml). After drying with anhydrous Na_2SO_4 and filtered, the CH_2Cl_2-soluble extract was evaporated to give DC-1E-8-12-ppt-Ac (a brown solid, 8.4 mg) and purified by a column of silica gel

60 (Merck, 0.063-0.200 mm, 0.7 g) using hexane/EtOAc (2:1, 1:1) as the eluent to give the acetate derivative (**D-3a**) as white needles (3.4 mg).

Acetylation of DC-1E-8-14 (**D-4**)

DC-1E-8-14 (**D-4**, a brown solid, 25.2 mg) was acetylated as described above to give DC-1E-10-14-Ac as a brown solid (28.4 mg). The brown solid was purified by a column of silica gel 60 (Merck, 0.063-0.200 mm, 0.7 g) using hexane/EtOAc (2:1, 1:1) as the eluent to give the acetate derivative (**D-4a**) as pale yellow needles (14.1 mg).

Fraction DC-1E-9

Fraction DC-1E-9 (a dark brown wax, 388.9 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 38 g) using CH_2Cl_2/MeOH/H_2O (300:3:1, 200:3:1, 100:3:1, 80:3:1, 50:3:1, 30:3:1, 20:3:1) as the eluent to give 12 fractions. (Table 30)

Table 30 Fractions obtained from DC-1E-9.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-9-1	5.0	a yellow wax
DC-1E-9-2	50.8	a pale brown solid
DC-1E-9-3	18.9	a yellow oil
DC-1E-9-4	14.0	a orange oil
DC-1E-9-5	7.9	a pink wax
DC-1E-9-6	11.4	a pink wax
*DC-1E-9-7	12.2	a pale pink solid
*DC-1E-9-8	79.1	a pale brown solid
*DC-1E-9-9	46.4	a pale brown solid
DC-1E-9-10	35.4	a brown oil
DC-1E-9-11	13.4	a brown semisolid
DC-1E-9-12	17.6	a brown semisolid

* Fractions were further investigated.

Fraction DC-1E-9-7 (a pale pink solid, 12.2 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 1.00 mm, 1/2 plate) using CH_2Cl_2/MeOH/H_2O (50:3:1, 5 runs) as the developing solvent to give DC-1E-9-7-1 as a cream solid (4.7 mg) which was identified as compound **D-3**.

Fraction DC-1E-9-8 (a pale brown solid, 79.1 mg) was precipitated by CH_2Cl_2 to give the precipitate (DC-1E-9-8-ppt) as a pale yellow solid (19.9 mg) which was identified to be compound **D-3** and the filtrate (DC-1E-9-8-f) as a brown wax (57.3 mg).

Fraction DC-1E-9-9 (a pale brown solid, 46.4 mg) was precipitated by $CHCl_3$ to give the precipitates (**D-3**, 18.8 mg) as a cream solid and the filtrate (DC-1E-9-9-f) as a brown wax (27.6 mg).

Fraction DC-1E-10

Fraction DC-1E-10 (a dark brown wax, 422.3 mg) was separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 40 g) using CH_2Cl_2/MeOH/H_2O (800:3:1, 500:3:1, 400:3:1, 300:3:1, 200:3:1, 100:3:1, 50:3:1) as the eluent to give 15 fractions. (Table 31)

Table 31 Fractions obtained from DC-1E-10.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-10-1	3.6	a yellow solid
DC-1E-10-2	11.5	a orange solid
DC-1E-10-3	9.5	a brown wax
DC-1E-10-4	8.9	a brown wax
DC-1E-10-5	11.0	a yellow wax
DC-1E-10-6	29.2	a brown wax
DC-1E-10-7	38.6	a brown wax
DC-1E-10-8	16.2	a brown wax
DC-1E-10-9	13.0	a orange wax
DC-1E-10-10	13.8	a yellow wax
*DC-1E-10-11	30.5	a brown solid
DC-1E-10-12	31.9	a brown solid
DC-1E-10-13	61.1	a brown wax
DC-1E-10-14	45.1	a brown oil
DC-1E-10-15	26.7	a brown oil

* Fractions were further investigated.

Fraction DC-1E-10-11 (a brown solid, 30.5 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 2 plates) using CH_2Cl_2/MeOH/H_2O (30:3:1, 1 run) as the developing solvent to give 2 fractions. (Table 32)

Table 32 Fractions obtained from DC-1E-10-11.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-10-11-1	4.9	a white solid
DC-1E-10-11-2	2.7	a pale yellow solid

DC-1E-10-11-1 (a white solid, 4.9 mg) was also characterized as compound **D-3**.

Fraction DC-1E-11 combined with fraction DC-1E-12

Fraction DC-1E-11 (a dark brown wax, 583.9 mg) and fraction DC-1E-12 (a dark brown wax, 592.0 mg) were combined and separated on a column of silica gel 60 (Merck, 0.063-0.200 mm, 115 g) using CH_2Cl_2/MeOH/H_2O (800:3:1, 500:3:1, 400:3:1, 300:3:1, 200:3:1, 150:3:1, 100:3:1, 50:3:1, 30:3:1, 20:3:1) as the eluent to give 15 fractions. (Table 33)

Table 33 Fractions obtained from DC-1E-1112.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-1112-1	25.3	a brown solid
DC-1E-1112-2	20.1	a brown wax
DC-1E-1112-3	23.1	an orange solid
*DC-1E-1112-4	49.7	a pale yellow solid
DC-1E-1112-5	23.0	a brown wax
DC-1E-1112-6	23.5	a brown wax
DC-1E-1112-7	25.7	a brown semisolid
DC-1E-1112-8	16.5	a brown semisolid
DC-1E-1112-9	33.0	a brown wax
DC-1E-1112-10	75.6	a brown wax
DC-1E-1112-11	117.7	a brown wax + a white solid
DC-1E-1112-12	108.5	a brown solid
DC-1E-1112-13	49.4	a brown solid
DC-1E-1112-14	64.0	a brown wax
DC-1E-1112-15	52.8	a brown solid

* Fractions were further investigated.

Fraction DC-1E-1112-4 (a pale yellow solid, 49.7 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.50 mm, 2 plates) using CH_2Cl_2/MeOH/isopropanol/H_2O (200:3:4:1, 2 runs) as the developing solvent to give 4 fractions (Table 34)

Table 34 Fractions obtained from DC-1E-1112-4.

Fraction No.	Weight (mg)	Physical characteristic
DC-1E-1112-4-1	9.8	a brown solid
DC-1E-1112-4-2	8.7	a pale yellow solid
*DC-1E-1112-4-3	10.3	a yellow wax
DC-1E-1112-4-4	13.7	a brown wax

* Fractions were further investigated.

DC-1E-1112-4-2 (8.7 mg) was identified as compound **D-5**.

DC-1E-1112-4-3 (a yellow wax, 10.3 mg) was separated on preparative TLC (silica gel 60 F_{254}, layer thickness 0.25 mm, 1 plate) using CH_2Cl_2/MeOH/ /H_2O (300:3:1, 4 runs) as the developing solvent to give DC-1E-1112-4-3-1 as a white solid (1.7 mg) and DC-1E-1112-4-3-2 as a pale yellow solid (**D-6**, 7.5 mg).

D-1

D-1 was obtained as a pale yellow solid; m.p. 141-143°C; $[\alpha]_D^{27}$ + 14.68° (*c* = 0.07, MeOH); UV λ_{max}^{MeOH} (log ε) nm : 203 (3.82) 237 (3.93) 279 (3.73); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1} : 3406, 2954, 2870, 1737, 1670, 1625, 1463, 1384, 1295, 1182, 1061, 948; ^{1}H–NMR ($CDCl_3$) : see **Table 35**; ^{13}C–NMR ($CDCl_3$) : see **Table 35**.

D-2

D-2 was obtained as colorless needles; m.p. 246°C; UV λ_{max}^{MeOH} (log ε) nm : 203 (4.15) 248 (4.03); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1} : 3417, 2923, 2851, 1735, 1607, 1514, 1456, 1378, 1266, 1089, 1027, 738; ^{1}H–NMR ($CDCl_3$) : see **Table 36**; ^{13}C–NMR ($CDCl_3$) : see **Table 36**.

D-3 and D-3a

D-3 was obtained as a cream solid; the acetate derivative (**D-3a**) was obtained as white needles; m.p. 132-134 °C; ^{1}H–NMR ($CDCl_3$): see **Table 37**; ^{13}C–NMR ($CDCl_3$): see **Table 37**.

D-4 and D-4a

D-4 was obtained as a brown solid; the acetate derivative (**D-4a**) was obtained as pale yellow needles; m.p. 163-165 °C; ^{1}H–NMR ($CDCl_3$): see **Table 38**; ^{13}C–NMR ($CDCl_3$): see **Table 38**.

D-5

D-5 was obtained as a pale yellow solid; m.p. 72-73 °C; $[\alpha]_D^{27}$ -79.33° (*c* = 0.11, MeOH); UV λ_{max}^{MeOH} (log ε) nm : 205 (4.66) 220 (4.59) 237 (4.22) 246sh (4.17) 293 (4.25); IR ν_{max}^{nujol} cm^{-1} : 3435, 2918, 1674, 1610, 1510, 1457, 1334, 1217, 1088, 1026, 819, 748, 657; HRTOFMS found : *m/z* 449.1210 $[M+Na]^+$; Calculated for $[C_{23}H_{22}O_8+Na]^+$: *m/z* 449.1212; ^{1}H–NMR ($CDCl_3$) : see **Table 39**; ^{13}C–NMR ($CDCl_3$) : see **Table 40**.

D-6

D-6 was obtained as a pale yellow solid; m.p. 72-73 °C; $[\alpha]_D^{27}$ + 74.68° (*c* = 0.07, MeOH); UV λ_{max}^{MeOH} (log ε) nm : 205 (4.72) 220 (4.55) 236 (4.26) 246sh (4.20) 294 (4.30); IR ν_{max}^{nujol} cm^{-1} : 3445, 2919, 1674, 1610, 1510, 1457, 1334, 1260, 1217, 1156, 1088, 1027, 818, 747, 663; HRTOFMS found : *m/z* 449.1214 $[M+Na]^+$; Calculated for $[C_{23}H_{22}O_8+Na]^+$: *m/z* 449.1212; ^{1}H–NMR ($CDCl_3$) : see **Table 39**; ^{13}C–NMR ($CDCl_3$) : see **Table 40**.

Table 35 ^{1}H- and ^{13}C-NMR spectral data of **D-1**.
(stigmasta-5, 22-dien-3β-ol-7-one,**10**)

position	δ_H	δ_C
1	1.93 (1H, m), 1.20 (1H, m)	36.4
2	1.93 (1H, m), 1.64 (1H, m)	31.1
3	3.67 (1H, m)	70.5
4	2.51 (1H, m), 2.40 (1H, m)	41.8
5	-	165.4
6	5.69 (1H, s)	126.0
7	-	202.6
8	2.25 (1H, br t, 10.8)	45.4
9	1.51 (1H, m)	49.9
10	-	38.3
11	1.57 (2H, m)	21.2
12	2.03 (1H, m), 1.13 (1H, m)	38.7
13	-	43.1
14	1.35 (1H, m)	50.0
15	1.28 (2H, m)	26.3
16	1.91 (1H, m), 1.30 (1H, m)	28.6
17	1.10 (1H, m)	54.7
18	0.68 (3H, s)	12.0
19	1.20 (3H, s)	17.3
20	2.03 (1H, m)	40.3
21	1.03 (3H, d, 6.6)	21.4
22	5.17 (1H, dd, 8.4, 15.0)	138.1

Table 35 ^{1}H- and ^{13}C-NMR spectral data of **D-1**. (continued)

(stigmasta-5, 22-dien-3β-ol-7-one, **10**)

position	δ_H	δ_C
23	5.02 (1H, dd, 8.4, 15.0)	129.4
24	1.53 (1H, m)	51.2
25	1.67 (1H, m)	29.1
26	0.83 (3H, d)	19.8
27	0.81 (3H, d)	19.0
28	1.30 (1H, m), 1.20 (1H, m)	23.0
29	0.84 (3H, t)	12.0

Table 36 ^{1}H- and ^{13}C-NMR spectral data of **D-2**. (formononetin, **11**)

position	δ_H	δ_C
2	7.96 (1H, s)	153.0
3	-	124.8
4	-	177.1
4a	-	117.5
5	8.10 (1H, d, 8.7)	127.9
6	6.94 (1H, dd, 2.1, 8.7)	115.6
7	-	163.0
8	6.86 (1H, d, 2.1)	102.7
8a	-	158.6
1’	-	124.5
2’, 6’	7.47 (2H, d, 8.7)	114.1 (2X)
3’, 5’	7.59 (2H, d, 8.7)	130.4 (2X)
4’	-	159.9
4’-O<u>C</u>H$_3$	3.85 (3H, s)	55.5

Table 37 ^{1}H- and ^{13}C-NMR spectral data of **D-3a**.
(4-acetoxy-3-methoxybenzoic acid, **12a**)

position	δ_H	δ_C
1	-	127.9
2	7.71 (1H, d, 1.8)	113.8
3	-	151.2
4	-	144.4
5	7.14 (1H, d, 8.1)	123.0
6	7.76 (1H, dd, 1.8, 8.1)	123.4
1-COOH	-	170.8
3-OCH_3	3.91 (3H, s)	56.1
4-O$COCH_3$	-	168.5
4-OCOCH_3	2.35 (3H, s)	20.7

Table 38 ^{1}H- and ^{13}C-NMR spectral data of **D-4a**. (4-acetoxybenzoic acid, **13a**)

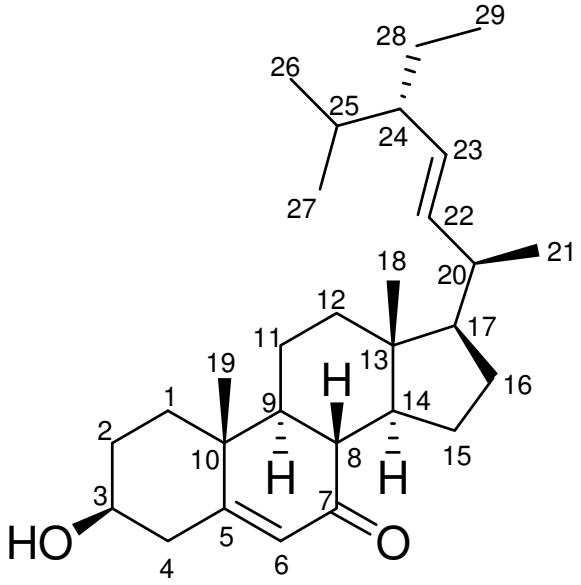
position	δ_H	δ_C
1	-	126.9
2, 6	8.16 (2H, d, 8.7)	121.8
3, 5	7.23 (2H, d, 8.7)	131.9
4	-	155.0
1-COOH	-	171.2
4-O$COCH_3$	-	168.8
4-OCOCH_3	2.35 (3H, s)	21.2

Table 39 ^{1}H-NMR spectral data of **D-5** and **D-6**.

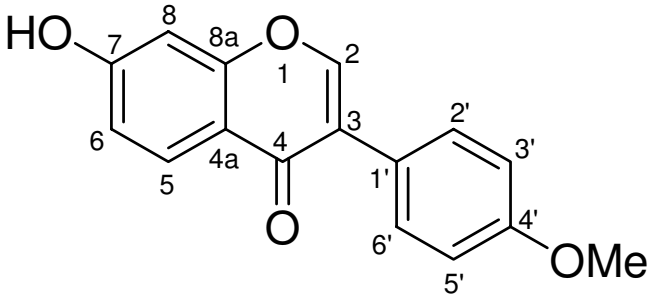
position	**D-5** [12aβ-hydroxyamorphiginin, **8**]	**D-6** [12aα-hydroxyamorphiginin, **9**]
1	6.58 (1H, s)	6.56 (1H, s)
4	6.50 (1H, s)	6.50 (1H, s)
6	4.50 (1H, dd, 1.2, 12.6), 4.62 (1H, dd, 1.2, 12.6)	4.50 (1H, dd, 2.4, 12.9), 4.62 (1H, dd, 2.4, 12.9)
6a	4.61 (1H, br s)	4.60 (1H, br s)
10	6.55 (1H, d, 8.4)	6.55 (1H, d, 8.4)
11	7.85 (1H, d, 8.4)	7.85 (1H, d, 8.4)
4’	3.07 (1H, dd, 8.7, 15.6), 3.36 (1H, dd, 9.9, 15.6)	3.07 (1H, dd, 8.4, 15.6), 3.36 (1H, dd, 9.9, 15.6)
5’	5.48 (1H, t, 9.0)	5.41 (1H, br t, 9.3)
7’	5.24 (1H, br s), 5.27 (1H, br s)	5.28 (1H, br s), 5.30 (1H, br s)
8’	4.24 (2H, br t, 13.5)	4.26 (2H, br t, 14.4)
2-O$\underline{C}H_3$	3.75 (3H, s)	3.74 (3H, s)
3-O$\underline{C}H_3$	3.82 (3H, s)	3.83 (3H, s)

Table 40 ^{13}C-NMR spectral data of **D-5** and **D-6**.

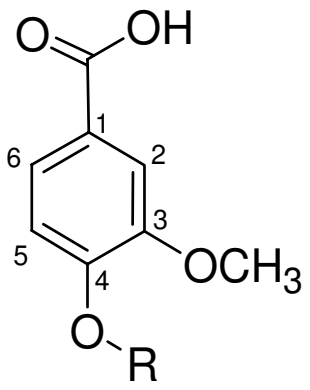
position	**D-5** [12aβ-hydroxyamorphiginin, **8**]	**D-6** [12aα-hydroxyamorphiginin, **9**]
1	109.3	109.3
2	144.0	144.0
3	151.1	151.1
4	101.1	101.1
4a	148.4	148.4
6	63.9	63.8
6a	76.3	76.0
7a	157.7	157.7
8	113.1	113.2
9	167.6	167.6
10	105.4	105.4
11	130.1	130.1
11a	108.7	108.6
12	191.1	191.1
12a	67.6	67.6
12b	112.0	111.9
4’	31.5	31.7
5’	85.6	85.7
6’	146.5	146.5
7’	112.8	112.8
8’	63.0	62.9
2-O$\underline{C}H_3$	56.4	56.4
3-O$\underline{C}H_3$	55.9	55.9



D-1

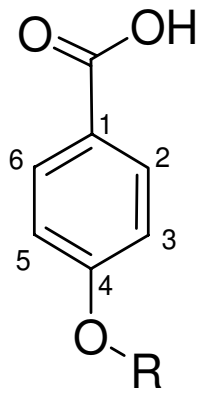


D-2



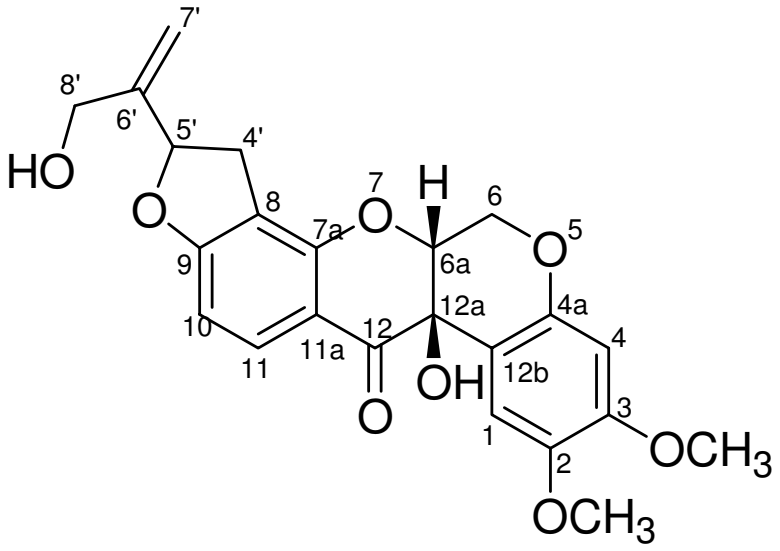
D-3 ; R = H

D-3a; R = Ac

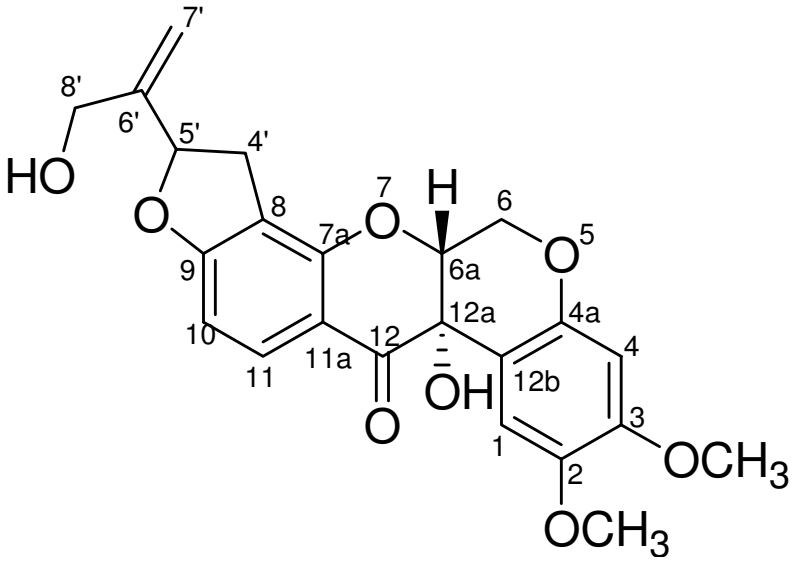


D-4 ; R= H

D-4a ; R= Ac



D-5

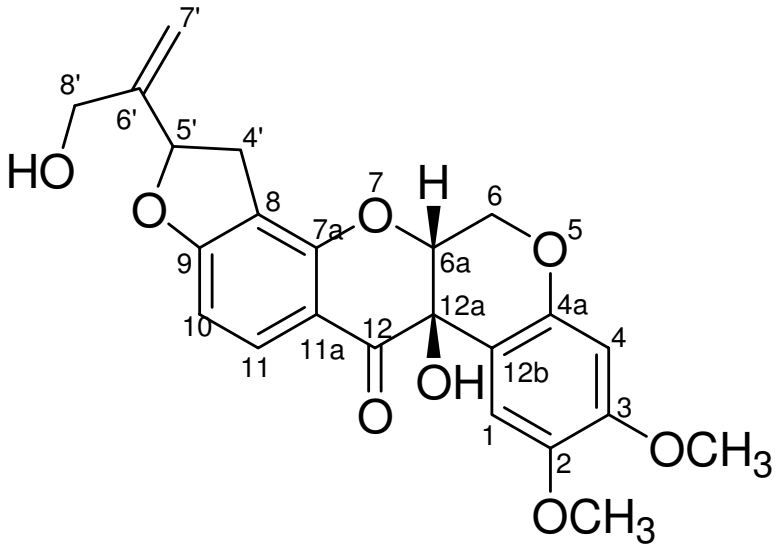


D-6

RESULT AND DISCUSSION

The crude EtOAc extracts from the branches of *D. cochinchinensis* Pierre were separated by chromatographic methods to yield one new compound, 12aα-hydroxyamorphiginin (**9**) together with five known compounds, 12aβ-hydroxyamorphiginin (**8**), stigmasta-5, 22-dien-3β-ol-7-one (**10**), formononetin (**11**), 4-hydroxy-3-methoxybenzoic acid (**12**) and 4-hydroxybenzoic acid (**13**). The structures were elucidated by spectroscopic analysis including 2D NMR techniques and by comparison of their spectral data with those previously reported in the literatures.

D-5



8 (12aβ-hydroxyamorphiginin)

D-5 was isolated as a pale yellow solid, m.p. 72-73 °C, which was shown to be optically active ($[\alpha]_D^{27}$ -79.33° (*c* = 0.11, MeOH)). The compound had the molecular formula $C_{23}H_{22}O_8$ by HRESIMS. The UV spectrum showed bands at 237, 246sh and 293 nm. The IR spectrum showed absorption bands for the hydroxyl (3435 cm^{-1}), the carbonyl (1674 cm^{-1}) and the C-O bond (1217 cm^{-1}). The signal of four aromatic protons at δ 6.55 (d, J = 8.4) 7.85 (d, J = 8.4), 6.50 (s) and 6.58 (s) in the ^{1}H-NMR spectrum of **D-5** (Table 39) were ascribed to one 1, 2, 3, 4-tetrasubstituted and one 1, 2, 4, 5-tetrastituted benzene ring. This was consistent with the ^{13}C-NMR spectral data (Table 40) which exhibited four aromatic methine carbons at δ 105.4 (C-10), 130.1 (C-11), 101.1 (C-1) and 109.3 (C-4) and eight quaternary aromatic carbons at δ 108.7 (C-11a), 157.7 (C-7a), 113.1 (C-8), 167.6 (C-9), 144.0 (C-2), 151.1 (C-3), 148.4 (C-4a) and 111.1 (C-12b). In addition, the

signal of two ABX systems in the ^{1}H-NMR spectrum of **D-5** at δ 4.50 (dd, J=1.2, 12.6 Hz), 4.62 (dd, J=1.2, 12.6 Hz) and 4.61 (br s) and 3.07 (dd, J=9.0, 15.6 Hz), 3.36 (dd, J=9.0, 15.6 Hz) and 5.48 (br t, J=9.0 Hz) were assigned to H_{ab}-6 and H-6a and H_{ab}-4' and H-5', respectively. This was consistent with the ^{13}C-NMR spectrum which showed peaks of two methylene carbons at δ 63.9 and 31.5 and two methine carbons at δ 76.3 and 85.6. The spectrum also contained an terminal olefinic function at δ 5.24 and 5.27 (both br s) and an oxymethylene group at δ 4.24 (br t, J=13.5 Hz), which was in good agreement with the ^{13}C-NMR spectrum, exhibiting signals of one olefinic methylene carbon at δ112.8, an oxymethylene carbon at δ 63.0 and an olefinic quaternary carbon at δ 146.5. The ^{13}C-NMR spectral data of **D-5** also contain a peak of ketocarbonyl at δ 191.1. The ^{13}C-NMR spectrum of **D-5** (Table 40) was assigned by DEPT, 2D HMQC and 2D HMBC spectrum.

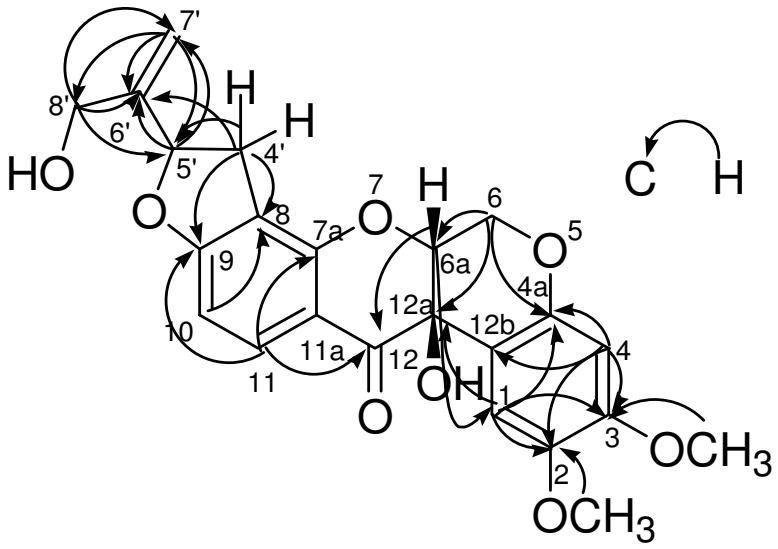
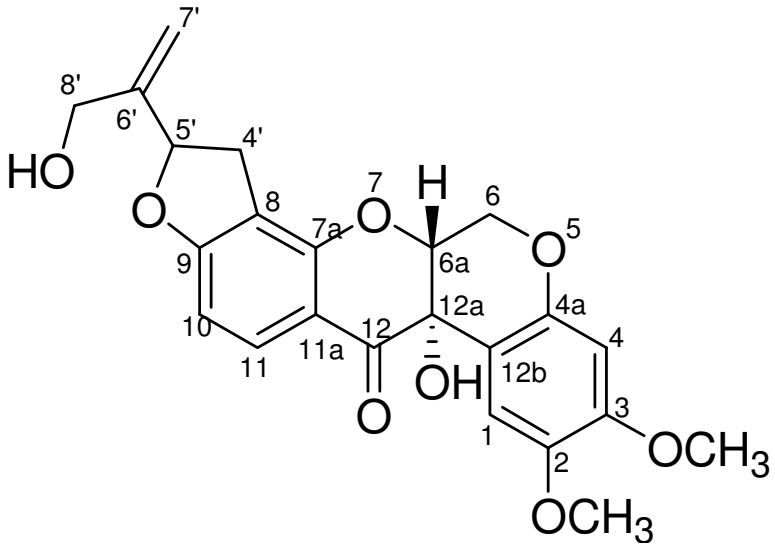


Figure 7 Selected 2D HMBC correlations of **D-5**.

Important HMBC correlations (Fig. 7) were observed. The methylene hydrogens at δ 4.50 and 4.62 (H_{ab}-6) showed correlations to C-6a (δ 76.3) and C-12a (δ 67.6). The aromatic protons at δ 6.58 (H-1) had correlations to C-12a, C-4a (δ 148.4), C-2 (δ 144.0) and C-3 (δ 151.1), and H-4 (δ 6.49) had correlations to C-12b (δ 111.1), C-2 (δ 144.0) and C-3 (δ 151.1). The carbonyl carbon at δ 191.1 (C-12) had 2D HMBC correlations with H-6a (δ 4.61) and H-11 (δ 7.85) and H-11 also showed correlations to C-7a (δ 157.7) and C-9 (δ 167.6). The methylene hydrogen at δ 3.07 and 3.36 (H_{ab}-4') had ^{3}J correlations to C-9 (δ 167.6). The terminal olefinic hydrogens (H_{ab}-7') showed 2D HMBC correlations to C-5' (δ 85.6) and C-8' (δ 63.0), while H_{ab}-8' showed correlations to C-5' (δ 85.6). On the basis of the above evidences and the negative optical rotation, compound **D-5** was thus identified as a known compound, 12aβ-hydroxyamorphiginin (**8**) [9].

6. **D-6**



9 (12aα-hydroxyamorphiginin)

D-6 was isolated as a pale yellow solid, m.p. 72-73 °C, which was shown to be optically active ($[\alpha]_D^{27}$ + 74.68° (*c* = 0.07, MeOH)). Its IR spectrum showed absorption bands corresponding to the stretching of the hydroxyl group at 3445 cm^{-1}, the carbonyl at 1674 cm^{-1} and the C-O bond at 1217 cm^{-1}. Its UV spectrum showed absorption band at λ_{max} 236, 246sh and 294 nm. The ^{1}H- and ^{13}C-NMR spectral data of **D-6** were very similar to those of **D-5** (Tables 39 and 40).

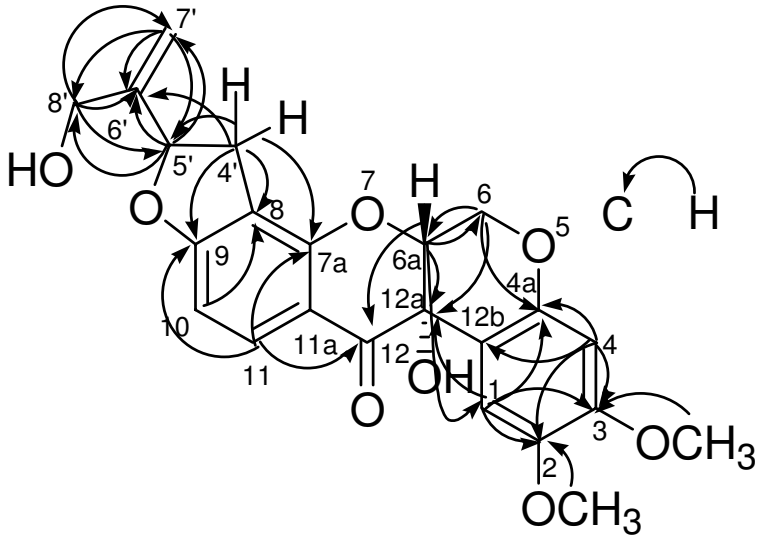
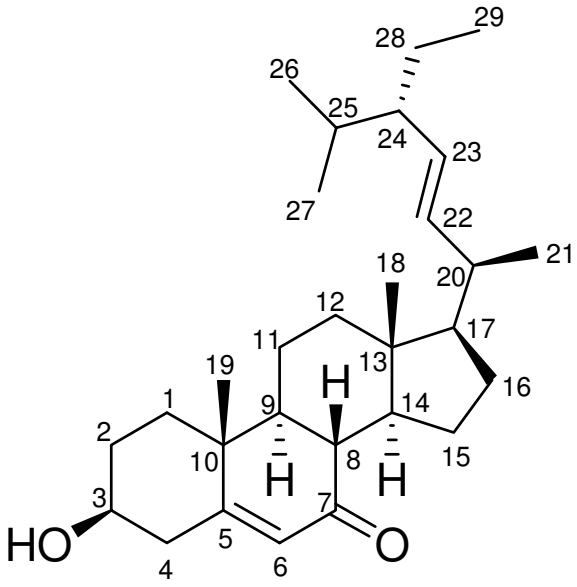


Figure 8 Selected 2D HMBC correlations of **D-6**.

The important HMBC correlations of **D-6** were also similar to those of **D-5** (Fig. 7-8). On the basis of the previous evidences and the positive optical rotation, **D-6** was characterized as a new compound, 12aα-hydroxyamorphiginin (**9**).

D-1



10 (stigmasta-5, 22-dien-3β-ol-7-one)

D-1 was isolated as a pale yellow solid, m.p. 141-143°C, which was shown to be optically active ($[\alpha]_D^{27.4}$ + 14.68 *c*, 0.07, MeOH). Its IR spectrum showed absorption bands corresponding to the stretching of the hydroxyl group at 3406 cm^{-1} and the carbonyl function at 1670 cm^{-1}. Its UV spectrum showed absorption peak at λ_{max} 203, 237 and 279 nm.

^{1}H-NMR spectrum of **D-1** (Table 35) contained a downfield olefinic proton at δ 5.69 (s, H-6) and two *trans* olefinic hydrogens appearing as two doublet of doublets of one hydrogen each at δ 5.17 (*J* = 8.4, 15.0 Hz, H-22) and 5.02 (*J* = 8.4, 15.0 Hz, H-23). The signals of two singlets of three hydrogens each at δ 0.68 (H-18) and 1.20 (H-19), three doublets of three hydrogens each at δ 1.03 (*J* = 6.6 Hz, H-21), 0.83 (H-26) and 0.81 (H-27) and a triplet of three hydrogens at δ 0.84

(H-29) were ascribed to six methyl groups in the molecule of **D-1**. This was consistent with the ^{13}C-NMR spectral data (Table 35), which exhibited six methyl carbons at δ 12.0 (C-18), 17.3 (C-19), 21.4 (C-21), 19.8 (C-26), 19.0 (C-27) and 12.0 (C-29). Seven methine protons (H-8, H-9, H-14, H-17, H-20, H-24 and H-25) and an oxymethine proton (H-3) (Table 35) were observed in the ^{1}H-NMR spectrum of **D-1**. This was in agreement with the ^{13}C-NMR spectral data which showed seven methine carbons at δ 45.4 (C-8), 49.9 (C-9), 50.0 (C-14), 54.7 (C-17), 40.3 (C-20), 51.2 (C-24) and 29.1 (C-25) and an oxymethine carbon at δ 70.5 (C-3). In addition, the ^{13}C-NMR spectrum of **D-1** (Table 35) showed eight methylene carbons at δ 36.4 (C-1), 31.1 (C-2), 41.8 (C-4), 21.2 (C-11), 38.7 (C-12), 26.3 (C-15), 28.6 (C-16) and 23.0 (C-28). This was consistent with the ^{1}H-NMR spectral data which exhibited eight methylene groups (16H) of H-1, H-2, H-4, H-11, H-12, H-15, H-16 and H-28 (Table 35). The ^{13}C-NMR spectrum also contained a carbonyl carbon at δ 202.6 (C-7), two quaternary carbons at δ 38.3 (C-10) and 43.1 (C-13) and an olefinic quaternary carbon at δ 165.4 (C-5) and three olefinic methine carbons at δ 126.0 (C-6), 138.1 (C-22) and 129.4 (C-23) (Table 35).

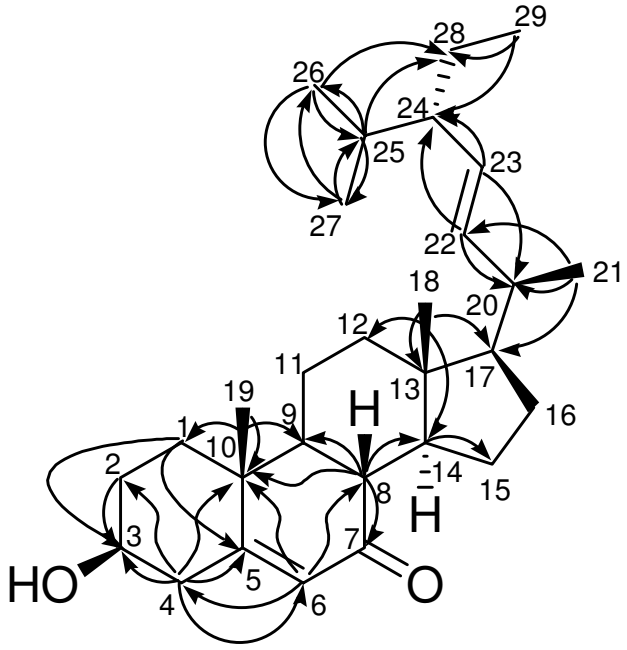
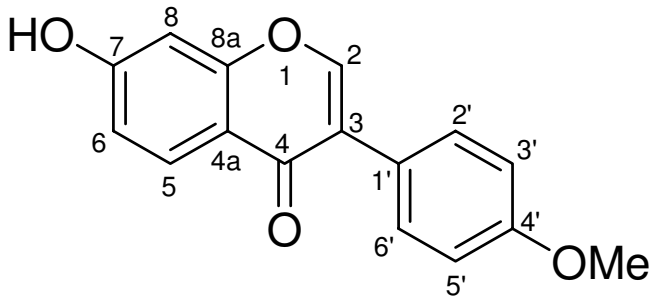


Figure 9 Selected 2D HMBC correlations of **D-1**.

Several important HMBC correlations were observed in the 2D HMBC spectrum of **D-1** (Fig. 9). The olefinic proton at δ 5.69 (H-6) showed correlations to C-4 (δ 41.8), C-8 (δ 45.4) and C-10 (δ 38.3) and H-8 (δ 2.25) had correlation to the carbonyl carbon at δ 202.6 (C-7). The HMBC correlations were observed between C-3 (δ70.5) and H-2 (δ 1.64 and 1.93) and H-4 (δ2.40 and 2.51). The olefinic proton at δ 5.17 (H-22) showed correlations to C-24 (δ 51.2) and C-20 (δ 40.3) and H-23 (δ 5.02) also had HMBC correlation to C-24 (δ 51.2) and C-20 (δ 40.3). The methyl protons at δ 1.03 (H-21) had correlation to C-17 (δ 54.7) and C-22 (δ 138.1). On the basis of the above evidences and by comparison with the previous literature data [10, 11] together with its melting point at 141-143°C (lit [10]. 144 °C), **D-1** was identified as stigmasta-5, 22-dien-3β-ol-7-one (**10**).

D-2



11 (formononetin)

D-2 was isolated as colorless needles, m.p. 246°C. Its IR spectrum showed absorption bands corresponding to the stretching of the hydroxyl group at 3417 cm^{-1}, the carbonyl at 1735 cm^{-1} and the C-O bond at 1266, 1027 cm^{-1}. Its UV spectrum showed absorption peak at λ_{max} 203 and 248 nm.

^{1}H-NMR spectrum (Table 36) of **D-2** contained three aromatic protons of one 1, 2, 4-trisubstituted aromatic ring appeared as a doublet of one hydrogen at δ 8.10 (*J* = 8.7 Hz, H-5), a doublet of doublets of one hydrogen at δ 6.94 (*J* = 2.1, 8.7 Hz, H-6) and a doublet of one hydrogen at δ 6.86 (*J* = 2.1 Hz, H-8), and four aromatic protons of one 1, 4-disubstituted aromatic ring appeared as two doublets of two hydrogens each at δ 7.47 (*J* = 8.7 Hz, H-2', H-6') and δ 7.59 (*J* = 8.7 Hz, H-3', H-5'). This was consistent with the ^{13}C-NMR spectral data of **D-2** (Table 36) which exhibited seven aromatic methine carbons at δ 127.9 (C-5), 115.6 (C-6), 102.7 (C-8), 114.1 (C-2', C-6') and 130.4 (C-3', C-5') and five quaternary aromatic carbons at 124.5 (C-1'), 159.8 (C-4'), 117.5 (C-4a), 163.0 (C-7) and 158.6 (C-8a). In addition, the signal of one olefinic proton at δ 7.96 (s, H-2) was

observed corresponding with the ^{13}C-NMR spectral data of **D-2** (Table 36) which showed the two olefinic carbons appeared at δ 153.0 (C-2) and 124.8 (C-3). Moreover, a singlet of three protons of one methoxyl group at δ 3.85 was also observed. This was in good agreement with the ^{13}C-NMR spectrum, exhibiting the signal of one methoxy carbon at δ 55.5. The ^{13}C-NMR spectral data of **D-2** (Table 36) also contained the one carbonyl carbon appeared at δ 177.1 (C-4). The ^{13}C-NMR spectral data of **D-2** were assigned by a combination of DEPT, 2D HMQC and 2D HMBC experiments.

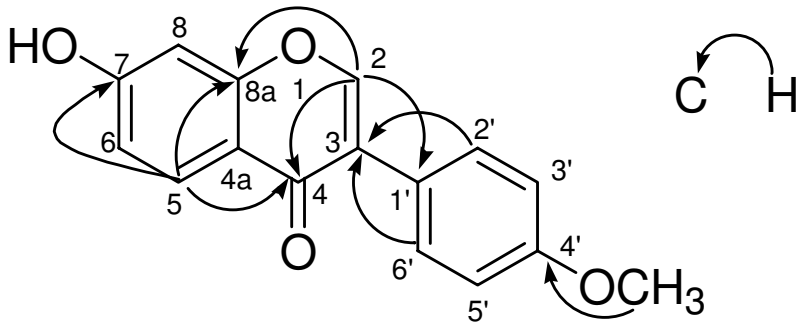
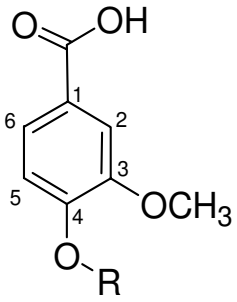


Figure 10 Selected 2D HMBC correlations of **D-2**.

Several important HMBC correlations were observed in the 2D HMBC spectrum of **D-2** (Fig. 10). The aromatic proton on the 1,2,4-trisubstituted aromatic ring at δ 8.10 (H-5) showed correlations to the carbonyl carbon at δ 177.1 (C-4) , aromatic carbons at δ 163.0 (C-7) and 158.6 (C-8a). The olefinic proton at δ 7.96 (H-2) had long-range correlations to aromatic carbons at δ 158.6 (C-8a), 124.5 (C-1') and the carbonyl carbon at δ 177.1 (C-4). The HMBC spectrum of **D-2** (Fig. 10) also showed correlations between the aromatic protons of 1, 4-disubstituted aromatic ring at δ 7.47 (H-2' and H-6') and C-3 at δ 124.8. In addition, the

HMBC correlation between the methoxy proton at δ 3.85 and the aromatic carbon at δ 159.8 (C-4') was also observed, implying that the methoxy group connected to the aromatic ring at C-4'. On the basis of the above evidences together with its melting point at 246 °C (lit. [12] 260 °C), **D-2** was characterized as formononetin (**11**).

D-3



D-3 ; R = H

D-3a; R = Ac

12 (4-hydroxy-3-methoxybenzoic acid); R= H

12a (4-acetoxy-3-methoxybenzoic acid); R= Ac

D-3 was isolated as a cream solid which was then acetylated to give **D-3a** as white needles, m.p. 132-134 °C. ^{1}H-NMR spectrum (Table 37) of **D-3a** contained three aromatic protons of the 1,3,4-trisubstituted aromatic ring appearing as two doublets of one hydrogen each at δ 7.71 (J = 1.8 Hz, H-2) and 7.14 (J = 8.1 Hz, H-5), and a doublet of doublets of one hydrogen at δ 7.76 (J = 1.8, 8.1 Hz, H-6). This was consistent with the ^{13}C-NMR spectral data (Table 37) which exhibited three aromatic methine carbons at δ 113.8 (C-2), 123.0 (C-5) and 123.4 (C-6) and three quaternary aromatic carbons at δ 127.9 (C-1), 151.2 (C-3) and 144.4 (C-4). In addition, an acetoxyl group appeared as a singlet at δ 2.35 (4-OCO$\underline{CH_3}$) and a singlet of one methoxyl group at δ 3.91 were observed. This was in good agreement with the ^{13}C-NMR spectrum, exhibiting signals of one methoxy carbon at δ 56.1 and one methyl carbon at δ 20.7 (4-OCO$\underline{CH_3}$). The ^{13}C-NMR spectral data of **D-3a** (Table 37) also contained one carbonyl carbon of acetoxy group appeared at δ 168.5 (4-O$\underline{CO}CH_3$), one carbonyl carbon of carboxylic group

appeared at δ 170.8 (1-COOH). The ^{13}C-NMR spectral data of **D-3a** were assigned by a combination of 2D HMBC experiments.

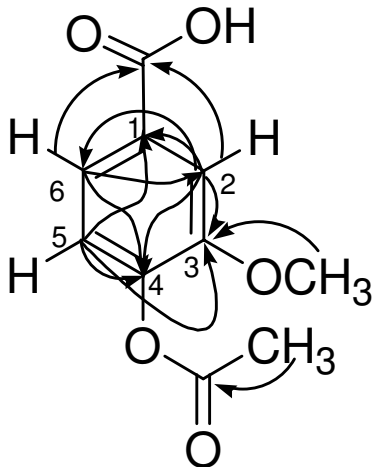
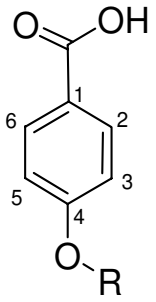


Figure 11 Selected 2D HMBC correlations of **D-3a**.

Important HMBC correlations were observed in the 2D HMBC spectrum of **D-3a** (Fig. 11). The aromatic protons at δ 7.71 (H-2) and 7.76 (H-6) had correlations to C-1 (δ 127.9), C-4 (δ 144.4) and carboxyl carbons at 170.8 (1-COOH). This indicated that the carbonyl group was at C-1. The methoxy proton at δ 3.91 showed correlations to aromatic carbons at δ 151.2 (C-3) implying that the methoxy group connected to the aromatic ring at C-3. In addition, the HMBC correlations on the aromatic ring were observed between the aromatic protons at δ 7.71 (H-2), 7.14 (H-5) and 7.76 (H-6) and the aromatic carbon at δ 144.4 (C-4) implying that the acetoxy group connected to the aromatic ring at C-4. On the basis of the above evidences together with its melting point at 132-134 °C (lit. [13] 143-144 °C), **D-3a** was identified as 4-acetoxy-3-methoxybenzoic acid (**12a**) and **D-3** was thus characterized as 4-hydroxy-3-methoxybenzoic acid (**12**).

4. **D-4**



D-4 ; R= H

D-4a ; R= Ac

13 (4-hydroxybenzoic acid); R = H

13a (4-acetoxybenzoic acid); R = Ac

D-4 was isolated as a brown solid which was then acetylated to give **D-4a** as pale yellow needles, m.p. 163-165 °C. ^{1}H-NMR spectrum (Table 38) of **D-4a** contained four aromatic protons appeared as two doublets of doublets of two hydrogens each at δ 8.16 (J = 8.7 Hz, H-2 and H-6) and 7.23 (J = 8.7 Hz, H-3 and H-5). In addition, a singlet of three protons of one acetoxymethyl group at δ 2.35 (4-OCO$\underline{CH_3}$) was also observed.

The ^{13}C-NMR spectral data of **D-4a** (Table 38) contained four aromatic methine carbons at δ 121.8 (C-2 and C-6) and 131.9 (C-3 and C-5), two quaternary aromatic carbons at δ 126.9 (C-1) and 155.0 (C-4) and one methyl carbon at δ 21.2 (4-OCO$\underline{CH_3}$) corresponding with the ^{1}H-NMR spectral data. In addition, one carbonyl carbon of the acetoxyl group appeared at δ 168.8 (4-O$\underline{CO}CH_3$) and one carbonyl carbon of the carboxylic group appeared at δ 171.2 (1-$\underline{CO}$OH). On the basis of the above evidence, **D-4a** were assigned as 4-acetoxybenzoic acid (**13a**) [14] and **D-4** was thus identified as 4-hydroxybenzoic acid (**13**).

REFERENCES

1. http://en.wikipedia.org/wiki/Dalbergia
2. http://www.unep-cmc.org/forest/timber/workshops/pdf/SEAsia_2007_V5.pdf
3. http://www.apforgen.org/pdf_files/InfoSheet_Dalbergia.pdf
4. D. M. X. Donnelly, B. J. Nangle, J. P. Prendergast and A. M. O'Sullivan. *Phytochemistry*, **7**(4), 647-649 (1968).
5. M. Kuroyanagi, A.Ueno, Y. Hirayama, Y. Hakamata, T.Gokita, T.Ishimaru, S. Kameyama, T. Yanagawa, M. Satake, and S. Sekita, *Natural Medicine*, **50**, 408 (1996).
6. V. Pathak, O. Shirota, S. Sekita, Y. Hirayama, Y. Hakamata, T. Hayashi, T. Yanagawa and M. Satake. *Phytochemistry*, **46**, 7, 1219-1223 (1997).
7. J. Svasti, C. Srisomsap, S. Techasakul and R. Surarit. *Phytochemistry*, **50**(5), 739-743 (1999).
8. T. K. Razdan, S. Harkar, B. Qadri, M. A. Khuroo. *Phytochemistry*, **27**, 1800 (1988).
9. S. S. Chibber, U. Khera. *Phytochemistry*, **17**, 1442-1443, 1978.
10. Y. Shua, S. R. Jonesa,b, W. A. Kinney and B. S. Selinskya. *Steroids*. **67**, 291–304 (2002).
11. P. Forgo and K. E. Kover. *Steroids*. **69**, 43–50 (2004).
12. H. M. T. B. Herath, R. S. Dassanayake, A. M. A. Priyadarshani, S. D. Silva, G. P. Wannigama and J. Jamie. *Phytochemistry*, **47**(1), 117-119 (1998).
13. C. Yuh-Lin, *Zhougguo Nongye Huaxue Huizhi*, 1-9 (1965).
14. M. Sunil, *Acta Ciencia Indica: Chemistry*, **28** (3), 131-134 (2002).

APPENDICES

δ	=	chemical shift relative to tetramethylsilane (TMS)
ε	=	molar absorptivity coefficient
λ_{max}	=	maximum wavelength
ν_{max}	=	absorption frequency
μm	=	micrometer
brs	=	broad singlet
d	=	doublet
dd	=	doublet of doublets
dq	=	doublet of quartets
$CDCl_3$	=	deuterochloroform
$CeSO_4$	=	cerium sulfate
CH_2Cl_2	=	dichloromethane
CH_3CN	=	acetonitile
COSY	=	correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
DPPH	=	2, 2-Diphenyl-1-picrylhydrazyl radical
EI MS	=	Electron-Ionization Mass Spectrometry
EtOAc	=	ethylacetate
eV	=	Electron Volt
g	=	gram
H_2SO_4	=	sulfuric acid
HMBC	=	Heteronuclear Multiple Bond Correlation

HMQC	=	Heteronuclear Multiple Quantum Coherence
HPLC	=	High Performance Liquid Chromatography
Hz	=	hertz
INEPT	=	Insensitive Nuclei Enhanced by Polarization Transfer
IR	=	Infrared
J	=	coupling constant
m	=	multiplet
MeOH	=	methanol
MIC	=	Minimum Inhibitory Concentration
MHz	=	Megahertz
mg	=	milligram
mL	=	mililiter
mm	=	millimeter
NMR	=	Nuclear Magnetic Resonance
NOE	=	Nuclear Overhauser Effect
NOESY	=	Nuclear Overhauser Enhancement Spectroscopy
PLC	=	Preparative Layer Chromatography
ppm	=	part per million
q	=	quartet
ROESY	=	Rotating frame Overhauser Enhancement Spectroscopy
RP	=	reverse phase
s	=	singlet
TLC	=	Thin Layer Chromatography
t	=	triplet
UV	=	Ultraviolet-Visible

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