# CHAPTER III

# EXPERIMENTAL

## 1. Sources of Plant Materials.

The rhizomes of *Belamcanda chinensis* (L.) DC. were purchased from Thai medicinal herb store in Bangkok, Thailand, in September 2003. The plant was identified by Prof. A Ueno of School of Pharmaceutical Sciences, University of Shizuoka. A voucher specimen, C003101, is deposited at Shizuoka.

The dried heartwood of *Dalbergia parviflora* Roxb. was purchased from Thai medicinal herb store in Bangkok, Thailand, in September 2003. The plant was identified by Dr. Chawalit Niyomdham of the Forest herbarium, Forest Botany Division, Royal Forest Department, Bangkok, Thailand. Authentication of the plant materials was done by Comparison with voucher specimens, BKF No. 68143, deposited in the Forest herbarium, Royal Forest Department, Bangkok, Thailand.

## 2. General Techniques.

### 2.1 Analytical Thin-Layer Chromatography (TLC).

| | | |
|---|---|---|
| Technique | : | One dimension, ascending |
| Adsorbent | : | Silica gel 60 $F_{254}$ (E. Merck) precoated plate (Aluminium sheet) |
| Layer thickness | : | 0.25 mm. |
| Distance | : | 5 cm. |
| Temperature | : | Laboratory temperature (25-35°c) |
| Detection | : | 1. Ultraviolet light (254 and 365 nm)<br>2. 50% $H_2SO_4$ in $H_2O$ and heating at 105°c for 10 min |

### 2.2 Column Chromatography.

#### 2.2.1 Vacuum Liquid Column Chromatography.

| | | |
|---|---|---|
| Adsorbent | : | Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck) |
| Packing method | : | Dry packing |

| | | |
|---|---|---|
| Sample loading | : | The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of absorbent, dried, triturated and then placed gently on top of the column |
| Detection | : | Fractions were examined by TLC observing under UV light (254 and 356 nm) and 50% $H_2SO_4$ spraying reagent in hot condition. |

**2.2.2 Gradient Formation Liquid Column Chromatography.**

| | | |
|---|---|---|
| Absorbent | : | Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck) |
| Packing method | : | Wet packing |
| Sample loading | : | The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of absorbent, dried, triturate and then placed gently on top of the column |
| Detection | : | Fractions were examine in the same manner as described in section 2.2.1 |

**2.2.3 Porus Polymer Gel Chromatography.**

| | | |
|---|---|---|
| Absorbent | : | Diaion HP-20 |
| Packing method | : | Wet packing |
| Sample loading | : | The sample was dissolved in water and then placed on top of the column. |
| Detection | : | Fractions were examine in the same manner as described in section 2.2.1 |

**2.2.4 High Performance Liquid Chromatography (HPLC).**

| | | |
|---|---|---|
| Column (Preparative) | : | Develosil-Lop-ODS No. 0603110, 10-20 μm, 5x100 cm (Nomura Chemical Co., Ltd.) |
| (Semi-prep.) | : | Capcell Pak ODS No. AQED 01063, 5μm, 2x25 cm (Shiseido Fine Chemical Co., Ltd.) |
| (Analytical) | : | Capcell Pak ODS, 5μm, 0.46x25 cm |
| Flow rate | : | 45 mL/min for semi-preparative column<br>6-9 mL/min for semi-preparative column<br>1 ml/min for analytical column |

| | | |
|---|---|---|
| Mobile phase | : | Acetonitrile (MeCN) in $H_2O$ |
| Sample preparative | : | The sample was dissolved in a small amount of eluent and injected into the column. |
| Injection volume | : | 2 g in 10 mL for preparative column<br>50 mg in 500 μL for semi-preparative column<br>1 mg in 10 μL for analytical column |
| Pump | : | 1. 887-PU (Jasco) for preparative column<br>2. 880-PU (Jasco) for semi-preparative and analytical column<br>3. PU-2089 Plus (Jasco) for semi-preparative column |
| Detector | : | UV-Vis detector 875-UV (Jasco) variable wavelength |
| Recorder | : | 1. Hitachi 561<br>2. Sekonic SS-250 F |
| Temperature | : | Room temperature |

**2.3 Gas chromatography (GC).**

| | | |
|---|---|---|
| Column | : | GL Sciences TC-1, 0.25 mm x30 m |
| Column temperature | : | 235 °C |
| Carrier gas | : | $N_2$ |

**2.4 Spectroscopy.**

**2.4.1 Ultraviolet (UV) Absorption Spectra.**

UV spectra were measured in MeOH with a Hitachi-U 3410 Spectrometer. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

**2.4.2 Proton and Carbon–13 Nuclear Magnetic Resonance ($^1$H and $^{13}$C-NMR) Spectra.**

$^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) Spectra were recorded on a JEOL JNM-α 400 instrument. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

Solvents for NMR spectra were deuterated dimethylsulfoxide (DMSO-$d_6$), deuterated chloroform ($CDCl_3$) deuterated acetone ($CD_3OCD_3$),

deuterated methanol ($CD_3OD$). Chemical shifts were given in $\delta$ (ppm) with tetramethylsilane (TMS) as an internal standard.

**2.4.3 Mass Spectra.**

FABMS were obtained on a JEOL JMS-700 mass spectrometer in *m*-nitrobenzyl alcohol (NBA) as the matrix agent. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

**2.5 Physical Properties.**

**2.5.1 Optical Rotations.**

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

**2.5.2 Circular Dichroism (CD) Spectra.**

CD Spectra were recorded on a JASCO J-20A spectropolarimeter. (School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan)

**2.6 Solvents.**

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

**2.7 Chemicals**

Eagle's MEM and RPMI media (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan)

Fetal bovine serum (FBS), trypsin-EDTA (Gibco, grand Island, NY, USA)

Antibiotics (Meiji Seika Kaisha Ltd., Tokyo, Japan)

L-Glutamine (Wako Pure Chemical Industries Ltd., Osaka, Japan)

17$\beta$-Estradiol, dextran-coated charcoal (DCC) (Sigma Chemicals, St. Louis, MO, USA)

OPTI-MEM Reduce Serum Medium, LIPOFECTAMINE$^{TM}$ Reagent (Invitrogen, CA, USA)

Pica Gene Luminescence Kit, Pica Gene Cell Culture Lysis Reagent LUC/PGC-50 (Toyo Ink Co., Tokyo, Japan)

**3. Extraction and Isolation.**

**3.1 Extraction and Isolation of Compounds from *Belamcanda chinensis*.**

**3.1.1 Extraction.**

The dried rhizomes of *Belamcanda chinensis* (1 Kg) were chopped, ground and then macerated with methanol (3x5 L). The extracts were combined and evaporated under reduced pressure at the temperature 60 ° C to yield 280 g (28 % base on dried weight of rhizomes) of yellow-brown gummy residue.

**3.1.2 Isolation.**

A part of this concentrated extract (140 g) was adsorbed on silica gel No. 7734 ( 400g ) and eluted successively with *n*-hexane (5 L), $CHCl_3$ (5 L), EtOAc (5 L) and MeOH (5 L), to yield a *n*-hexane soluble extract (9.5 g , 1.9 % of dried weight of rhizomes), a chloroform soluble extract (25.5 g, 5.1 % of dried weight of rhizomes), an ethyl acetate soluble extract (9 g, 1.8 % of dried weight of rhizomes) and a MeOH soluble extract (70.5 g , 14.1 % of dried weight of rhizomes) after evaporation of organic solvents.

The ethyl acetate soluble extract was chromatographed on a silica gel column (3.5 x 20 cm, silica gel 60 No.7734) and fractionated using chloroform-methanol (85:15, 15 L). Fractions of 300 ml were collected and pooled by TLC analysis to afford a total of 12 combined fractions: fraction I (85 mg), II (850 mg), III (520 mg), IV (1.28 g), V (550 mg), VI (485 mg), VII (680 mg), VIII (580 mg), IX (780 mg), X (1.7 g), XI (2.8 g), XII (260 mg) as shown in Scheme 4.

**3.1.2.1 Isolation of Compound BC1 (Tectorigenin) and BC3 (Irigenin)**

Fraction II (850 mg) was purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detector at 205 nm) with MeCN-$H_2O$ (30:70) as eluent to give BC1 (90 mg) and BC3 (115 mg) ($t_R$ = 228 and 272 min, respectively). Compounds BC1 and BC3 were identified as tectorigenin **[14]** and irigenin **[5]** , respectively.

### 3.1.2.2 Isolation of Compound BC2 (Irisflorentin).

Fraction I (85 mg) was purified by HPLC on Capcell Pak ODS (2 x 25 cm , flow rate 6 mL/min with detection at 205 nm) with MeCN-$H_2O$ (45:55) as eluent to give BC2 (59 mg) ($t_R$ = 41 min). It was identified as irisflorentin **[7]**.

### 3.1.2.3 Isolation of Compound BC4 (Irilin D).

Fraction III (520 mg) was purified by HPLC on Developsil-Lop-ODS (5 x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (27:73) as eluent to give BC4 (4 mg) ($t_R$ = 208 min). This compound was subsequently identified as irilin D **[412]**.

### 3.1.2.4 Isolation of compound BC5 (Tectoridin), BC6 (Iristectorin B) BC8 (Iridin), BC9 (Hispiduloside) and BC10 (Jaceoside).

Fraction VII (680 mg) was separated by HPLC using Developsil- Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (15:85) as eluent to give BC5 (96.1 mg), BC6 (155.5 mg), BC8 (283.3 mg), BC9 (20.9 mg) and BC10 (11.2 mg) ($t_R$ = 372, 452, 692, 800, and 864 min, respectively). Compounds BC5, BC6, BC8, BC9 and BC10 were identified as tectoridin **[13]**, iristectorin B **[413]**, iridin **[4]**, hispiduloside **[415]** and jaceoside **[416]**, respectively.

In addition, purification of fraction XI (2.8 g) using HPLC on Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (18:82) as eluent also gave tectoridin (1.1 g) ($t_R$ = 212 min).

### 3.1.2.5 Isolation of Compound BC7 (Iristectorin A).

Fraction V (550 mg) was purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (18:82) as eluent to give BC7 (30 mg) ($t_R$ = 432 min). This compound was later identified as iristectorin A **[414]**.

### 3.1.2.6 Isolation of Compound BC11 (Androsin), BC12 (Iriflophenone), BC13 (Belamphenone), BC14 (Belalloside A), BC15 (Belalloside B) and BC16 (Resveratrol).

Fraction IV (1.28 g ) was separated by HPLC using Developsil Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (18:82) as eluent to give BC11 (9.7 mg), BC12 (3.9 mg), BC13 (3.6 mg), BC14 (1.5 mg), BC15 (1.5 mg) and BC16 (385 mg) ($t_R$ = 164, 168, 188, 304, 312 and 476 min, respectively). Compound BC11 was identified as androsin **[417]**. Compound BC12 was identified as iriflophenone **[418]**. Compounds BC13, BC14 and BC15 were assigned as 1-(4-hydroxyphenyl)-2-(3,5-dihydroxphenyl) ethanone **[419]**, acetovanillone 1-*O*-*β*-D-(6-*O*-vanilloyl) glucopyranoside **[420]** and acetovanillone 1-*O*-*β*-D-(6-*O*-4-hydroxybenzoyl) glucopyranoside **[421]**, and given the trivial name as belamphenone, belalloside A and belalloside B, respectively. These compounds were isolated from natural sources for the first time in this study. Compounds BC16 was identified as resveratrol **[57]**.

## 3.2 Extraction and Isolation of Compounds from the Heartwood of *Dalbergia parviflora.*

### 3.2.1 Extraction

The dried coarsely powdered heartwood of *Dalbergia parviflora* (2 kg) was refluxed with methanol (3x20 L). The extracts were combined and evaporated to yield 910 g (45.5% of dried weight of heartwood) of a red-brown viscous mass.

### 3.2.2 Isolation.

A part of methanol extract (150 g) was then chromatographed on a silica gel column (12x40 cm, silica gel 60 No. 7734) and fractionated using a stepped gradient of chloroform and methanol (98:2, 96:4, 94:6, 9:1, 15 L each). Fractions of 500 mL were collected and grouped by TLC analysis to afford a total of 26 combined fractions: fraction A (3.1 g), B (0.5 g), C (0.6 g), D (0.6 g), E (0.5 g), F 3.1 g), G (3.2 g), H (4.3 g), I (8.5 g), J (9.9 g), K (1.6 g), L (0.9 g), M (8.9 g), N (8.9 g), O (4.9 g), P (5.2 g), Q (5.2 g), R (5.5 g), S (8.2 g), T (7.6 g), U (10.0 g), V (8.0 g), W (3.1 g), X (3.1 g), Y (4.2 g) and Z (9.6 g).

**3.2.2.1 Isolation of Compounds DP1 (Khriol A), DP2, (Mucronulatol), DP3 (7-De-*O*-methoxybustigenin), DP4 (3'-Methoxyviolanone), DP5 (Onogenin), DP6 (Sativanone), DP7 (Khrinone D), DP8 (Pinocembrin), DP9 (Biochanin A), DP10 (Hydroxyobustyrene) and DP11 (2'-Methoxybiochanin A).**

Fraction I (8.5g) was purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to give DP1 (140 mg)($t_R$ = 130 min), DP2 (870 mg)($t_R$ = 167 min), DP3 (8 mg)($t_R$ = 190 min), DP4 (3.8 g)($t_R$ = 210 min), DP5 (130 mg)($t_R$ = 220 min), DP6 (310 mg)($t_R$ = 240 min), DP7 (5 mg)($t_R$ = 270 min), DP8 (85 mg)($t_R$ = 307 mg), DP9 (320 mg)($t_R$ = 328 min), DP10 (55 mg)($t_R$ = 340 min) and DP11 (45 mg)($t_R$ = 488 min). Compound DP1 was assigned as khriol A **[422]**, which was isolated from natural sources for the first time in this study.

Compounds DP2, DP3, DP4, DP5 and DP6 were identified as mucronulatol **[77]**, 7-demethoxybustigenin **[423]**, 3'-methoxyviolanone **[197]**, onogenin **[252]**, and sativanone **[175]**, respectively. Compound DP7 was a novel compound and was confirmed as khrinone D **[424]**. Compounds DP8, DP9, DP10 and DP11 were pinocembrin **[174]**, biochanin A **[95]**, hydroxyobtustyrene **[355]** and 2'-methoxybiochanin A **[425]**, respectively.

**3.2.2.2. Isolation of Compounds DP12 ((6a*R*, 11a*R*)-3,8-dihydroxy-9-methoxypterocarpan), DP13 (8-Demethylduartin), DP14 (Pinobanksin) and DP15 (Secundiflorol H).**

Fraction J (9.9 g) was separated by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to yield 14 fractions: fraction J-a (30 mg), J-b (104 mg), J-c (540 mg), J-d (5,693 mg), J-e (320 mg), J-f (406 mg), J-g (1,330 mg), J-h (131 mg), J-i (57 mg), J-j (37 mg), J-k (42 mg), J-l (74 mg), J-m (64 mg) and J-n (359 mg).

Fraction J-c was further purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (45:55) as eluent to give DP12 (36 mg)($t_R$ = 75 min) and DP13 (113 mg)($t_R$ = 105 min). Compounds DP12 and DP13 were identified as (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan **[426]** and 8-demethylduartin **[427]**, respectively.

Fraction J-d was further purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 min) with MeOH-$H_2O$ (45:55) as eluent to give DP14 (800 mg)($t_R$ = 157 min) and DP15 (2.3 g)($t_R$ = 189 min). These compounds were identified as (+)-pinobanksin **[428]** and (-)-secundiflorol H **[429]**, respectively.

### 3.2.2.3 Isolation of Compound DP17 (Violanone).

Fraction N (8.9 g) was separated by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to give 14 fractions: N-a (7 mg), N-b (80 mg), N-c (210 mg), N-d (390 mg), N-e (3,500 mg), N-f (206 mg), N-g (1,114 mg), N-h (850 mg), N-i (46 mg), N- (20 mg), N-k (247 mg), N-l (22 mg), N-m (315 mg) and N-n (1,395 mg).

Fraction N-e further purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 min) with MeCN-$H_2O$ (30:70) as eluent to give DP17 (3,170 mg)($t_R$ = 204 min). It was identified as violanone **[177]**.

### 3.2.2.4 Isolation of Compound DP16 (7,3′-Dihydroxy-4′-methoxyisoflavanone).

Fraction N-d (390 mg) was separated on HPLC (Capcell Pak ODS 2x25 cm, flow rate 6 mL/min with detection at 205 nm) with MeCN-$H_2O$ (30:70) as eluent to give DP16 (125 mg)($t_R$ = 164 min). It was identified as 7,3′-dihydroxy-4′-methoxyisoflavanone **[430]**.

### 3.2.2.5 Isolation of Compounds DP18 (Arizonicanol A), DP19 (Tectorigenin), DP20 (Khrinone C) and DP21 (Vestitone).

Fraction N-g (1,114 mg) was further purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (25:75) as eluent to give DP18 (45 mg)($t_R$ = 197 min), DP19 (610 mg)($t_R$ = 220 min), DP20 (150 mg)($t_R$ = 228 min) and DP21 (25 mg)($t_R$ = 444 min). Compounds DP18 and DP19 were identified as arizonicanol A **[431]** and tectorigenin **[14]**, respectively. Compound DP20 was assigned as a new isoflavone, khrinone C **[432]** and compound DP21 was identified as vestitone **[176]**.

### 3.2.2.6 Isolation of Compound DP22 (Pratensein).

Fraction N-h (590 mg) was further purified by HPLC (Developsil-Lop-ODS 5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (30:70) as eluent to give DP22 (715 mg)($t_R$=371 min). This compound was identified as pratensein **[433]**.

### 3.2.2.7 Isolation of Compound DP23 (2′-Methoxyformononetin).

Fraction N-i (46 mg) was further purified by HPLC recycling mode (Capcell Pak ODS 2x25 cm, flow rate 9 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to give DP23 (10 mg)($t_R$ = 47.5 min). It was identified as 2′-methoxyformononetin **[434]**.

### 3.2.2.8 Isolation of Compounds DP24 (Formononetin) and DP25 (Vestitol).

Fraction N-k (247 mg) was further purified by using Capcell Pak ODS (2x25 cm, flow rate 9 mL/min with detection at 205 nm) recycling with MeCN-$H_2O$ (35:65) as eluent to give DP24 (30 mg)($t_R$ = 38.5 min) and DP25 (150 mg)($t_R$ = 40 min), respectively. Compound DP24 was identified as formononetin **[72]**. Compound DP25 was identified as vestitol **[78]**.

### 3.2.2.9 Isolation of Compound DP26 (Xenognosin)

Fraction N-m (315 mg) was further purified by HPLC using Capcell Pak ODS (2x25 cm, flow rate 9 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to give DP26 (15 mg)($t_R$ =112 min). It was identified as xenognosin **[435]**.

### 3.2.2.10 Isolation of Compounds DP27 (Dalparvinol A), DP28 (Khrinone B), DP29 (Dalparvin) and DP30 (5′-Methyxyvestitol).

Fraction O (4.9 g) was separated by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (30:70) as eluent to give DP27 (22 mg)($t_R$ = 74 min), DP28 (980 mg)($t_R$ = 106 min), DP29 (520 mg)($t_R$ = 188 min) and DP30 (140 mg)($t_R$ = 240 min), respectively.

Three new compounds DP27, DP28 and DP29 were isolated from this fraction. Compound DP27, which was a new 2,3-dihydroflavonol, (2*R*,3*R*)-(-)-dalparvinol A **[436]**. Compounds DP28 and DP29 were assigned as khrinone B **[437]** and dalparvin **[438]**, respectively. Compound DP30 was identified as (3*R*)-(-)-5′-methoxyvestitol **[183]**.

### 3.2.2.11 Isolation of Compound DP31 (Khrinone A).

Fraction P (5.2 g) was separated by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to give DP31 (406 mg)($t_R$ = 80 min). It was identified as khrinone A **[439]**.

### 3.2.2.12 Isolation of Compounds DP32 (Dalparvinol B), DP33 (Khrinone E), DP34 (3′-Methoxydaidzein), DP35 (Calycosin).

Fraction Q (5.2g) was chromatographed by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (30:70) as elute to give 12 fractions: Q-a (70 mg), Q-b (612 mg), Q-c (68 mg), Q-d (1.3g), Q-e (118 mg), Q-f (45 mg), Q-g (33 mg), Q-h (1.5g), Q-i (126mg), Q-j (227 mg), Q-k (964 mg) and precipitation Q′(57 mg).

Fraction Q-d (1.3 g) was further separated by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (25:75) as eluent to yield 7 fractions. Major fraction Q-d5 (965 mg) was further separated by HPLC (Developsil-Lop-ODS 5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (20:80) to give DP32 (10 mg)($t_R$ = 170 min). It was confirmed as a new 2, 3-dihydroflavonol, dalparvinol B **[440]**.

Compound DP35 (Calycosin **[81]**, 513 mg) was separated ($t_R$ = 497 min). Repeated purification of fraction Q-d54 (25 mg) by HPLC using Capcell Pak ODS (2x25 cm, flow rate 6 mL/min with detection at 205 nm) with MeOH-$H_2O$ (38:62) as eluent to afford 2 mg of DP33 ($t_R$ = 78 min) and 2 mg of DP34 ($t_R$ = 83 min). Compound DP33 was identified as new isoflavone, khrinone E **[441]**. And DP34 was identified as 3′-methoxydaidzein **[196]**.

### 3.2.2.13 Isolation of Compound DP36 (Theralin).

Fraction Q-g (33 mg) was chromatographed by HPLC using Capcell Pak ODS (2x25 cm, flow rate 6 mL/min with detection at 205 nm) with MeCN-$H_2O$ (22:78) as eluent to give DP36 (5 mg)($t_R$ = 165 min). It was identified as theralin **[442]**.

### 3.2.2.14 Isolation of Compounds DP37 (Naringenin) and Compound DP38 (Genistein).

Fraction Q-h (1.5g) was further purified by HPLC using Developsil-Lop-ODS (5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (25:75) as eluent to give DP37 (792mg)($t_R$=184 min) and DP38 (198mg)($t_R$ = 194 min), respectively. Compounds DP37 and DP38 were identified as naringenin **[251]** and genistein **[3]**, respectively.

### 3.2.2.15 Isolation of Compounds DP39 (Liquiritigenin) and Compound DP40 (Isoliquiritigenin).

Fraction Q-j (227mg) was purified by HPLC using Developsil-Lop-ODS(5x100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN-$H_2O$ (35:65) as eluent to give DP39 (32mg)($t_R$ = 22 min) and DP40 (116mg)($t_R$ = 50 min). Compound DP39 was identified as liquritigenin **[84]**. Compound DP40 was identified as isoliquiritigenin **[83]**.

### 3.2.2.16 Isolation of compound 41 (Bowdichione).

Fraction Q'(57mg) was purified by HPLC using Capcell Pak ODS (2x25 cm, flow rate 9 mL/min with detection at 205 nm) with MeCN-$H_2O$ (16.5:83.5) as eluent to yield DP41 (3mg)($t_R$ = 110 min). This compound was later identified as bowdichione **[443]**.

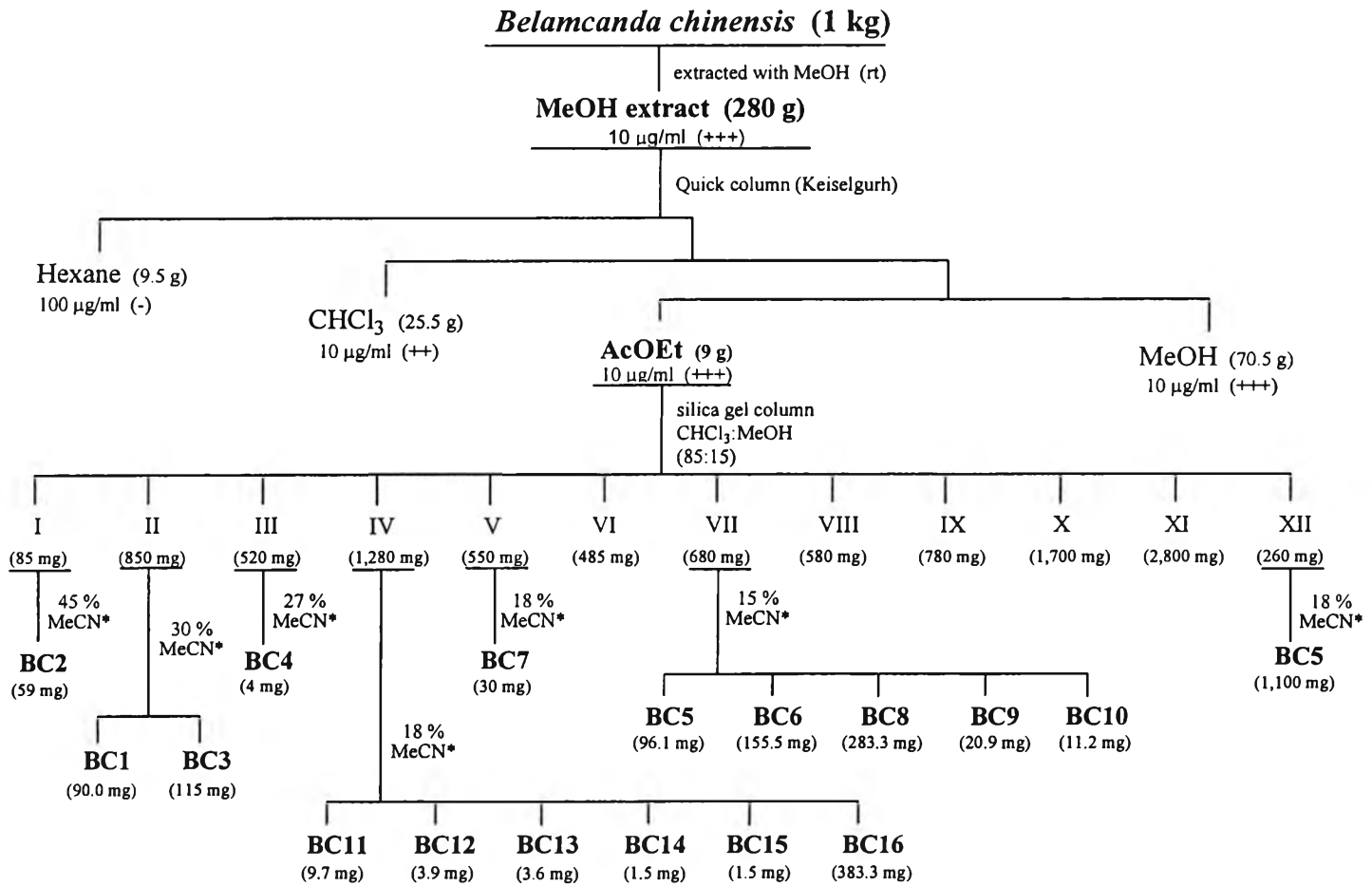
## 4. Acid Hydrolysis of BC 14 and BC 15.

Compound BC 14 (1 mg.) was dissolved in 10% $H_2SO_4$ (1 mL) and heated at 95 °C for 1 h. After cooling, the reaction mixture was diluted with $H_2O$ (2 mL) and extracted with ethyl acetate (2 mL × 3). The ethyl acetate phases were evaporated, and acetovanillone and vanillic acid were identified by direct comparison with authentic samples. The water layer was passed through an Amberlite IRA-60E

column (6×60 mm), and the eluate was concentrated. The residue was dissolved in pyridine (50μL) and stirred with D-cysteine methyl ester (3 mg) for 1.5 h. at 60 °C. To the reaction mixture, hexamethyldisilazane (15 μL) and trimethylsilyl chloride (15 μL) were added, and the mixture was stirred for 30 min at 60 °C. The supernatant was then analyzed by GC [column: GL Sciences TC-1, 0.25 mm x 30 m; column temperature: 235 °C; carrier gas: $N_2$; retention time: D-Glc (21.4 min), L-Glc (20.4 min) (Eddarir *et al.*, 2001)], and from BC 14, D-Glc was detected. Acid hydrolysis of BC 15 was performed in the same manner, and D-Glc was detected.

**5. Acetylation of DP28 and DP31.**

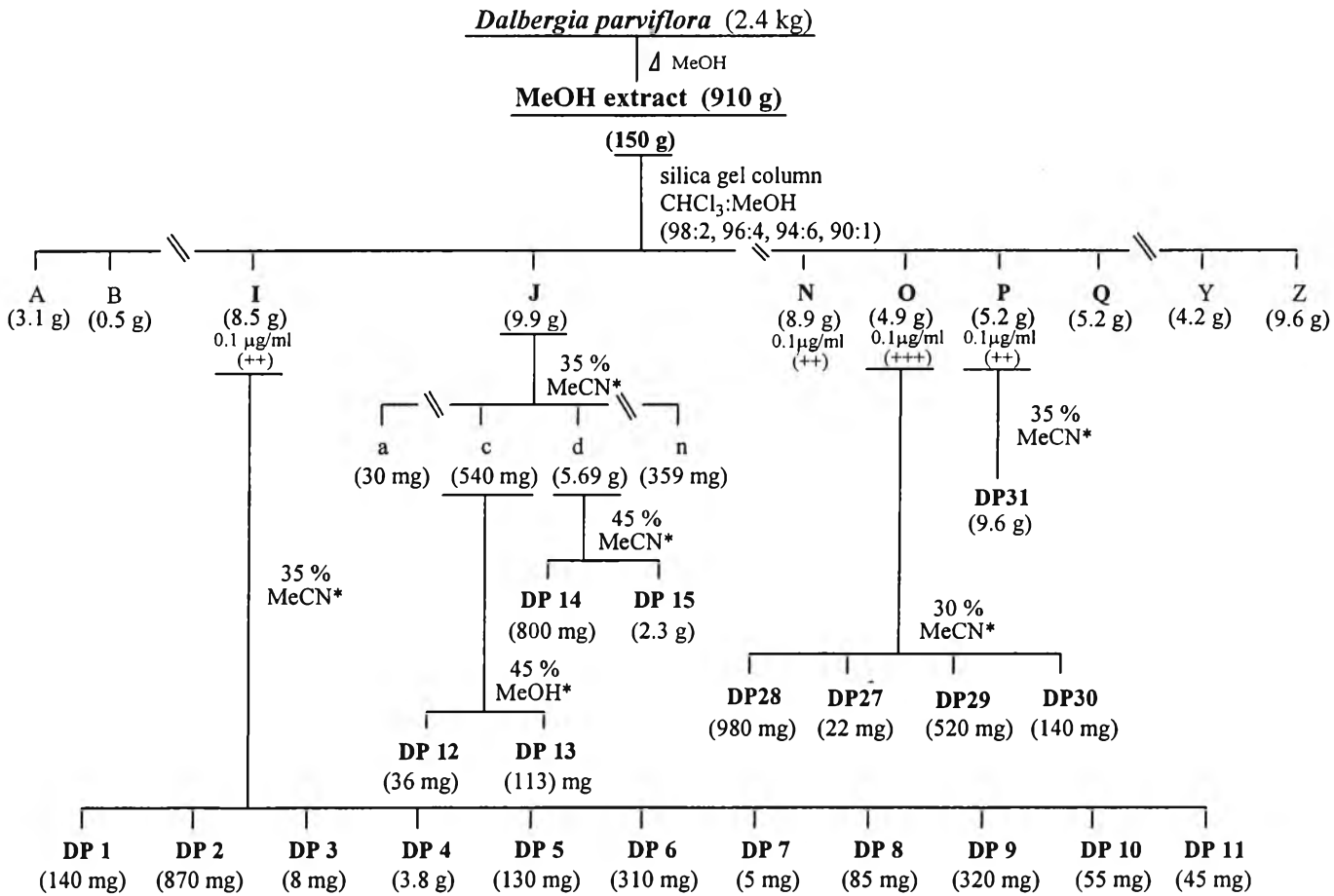
Compound DP28 (10 mg) and DP31 (10mg) were dissolved in 2-3 drops of pyridine and added 2-3 drops of acetic anhydride, and mixture were stirred at room temperature overnight. Then the solvents were evaporated off by using jet evaporator to give the acetates DP28a (12 mg) and DP31a (12 mg).




* Isolation overed HPLC preparative column: Capcell-pak ODS (2x25 cm), Solvent: 15-45% $CH_3CN$

Cell proliferation stimulative activities of samples: (+++),>100 pM of Estradiol(E2); (++), > 10 pM of E2; (+), > 1 pM; (-), < 1 pM of E2.

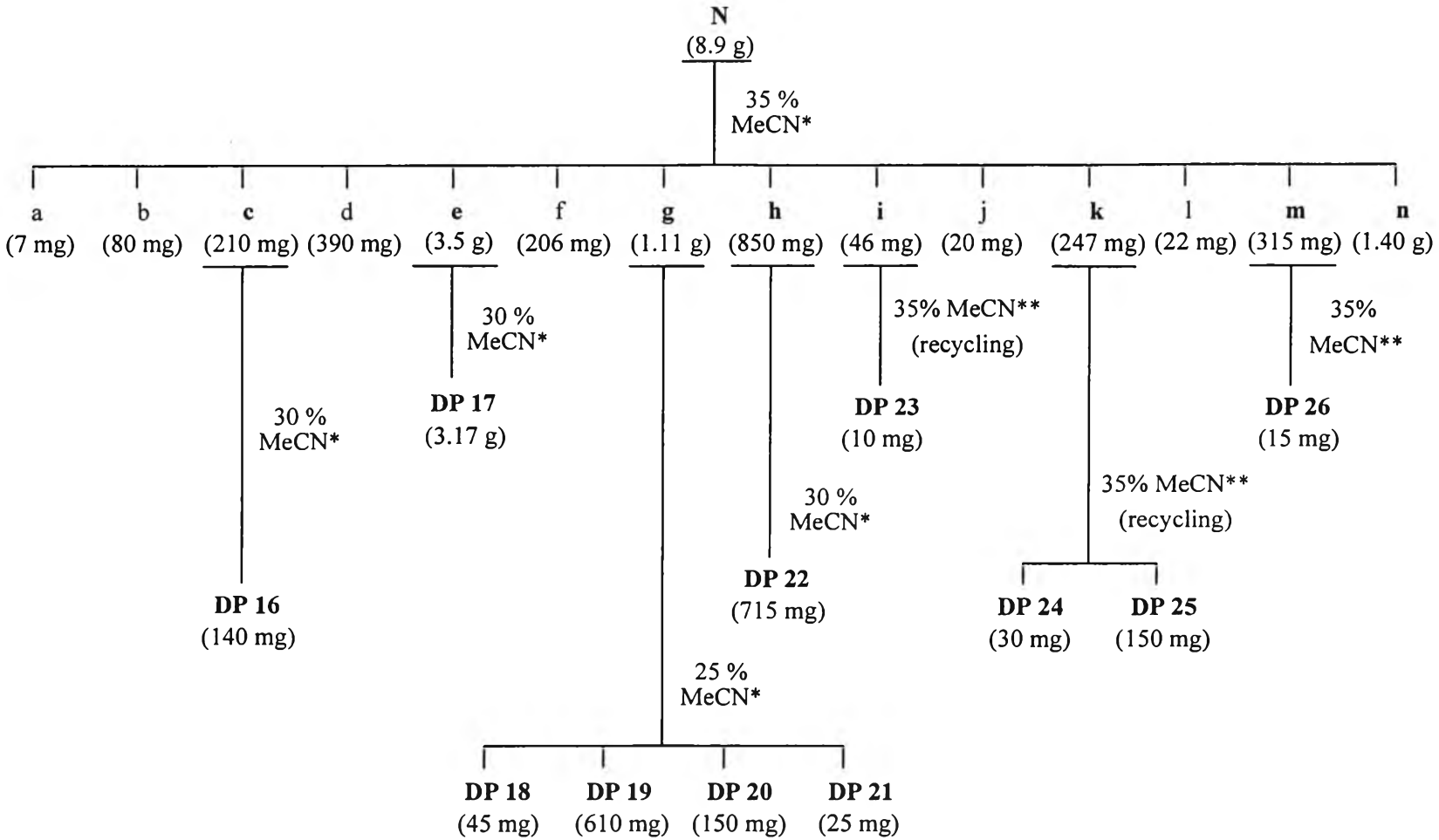
**Scheme 4** Separation of the rhizomes of *Belamcanda chinensis*




* Isolation overed HPLC preparative column: Developsil-Lop-ODS (5x100 cm), Solvent: 30-45% $CH_3CN$, 45% $CH_3OH$

Cell proliferation stimulative activities of samples: (+++),>100 pM of Estradiol(E2); (++), > 10 pM of E2; (+), > 1 pM; (-), < 1 pM of E2.

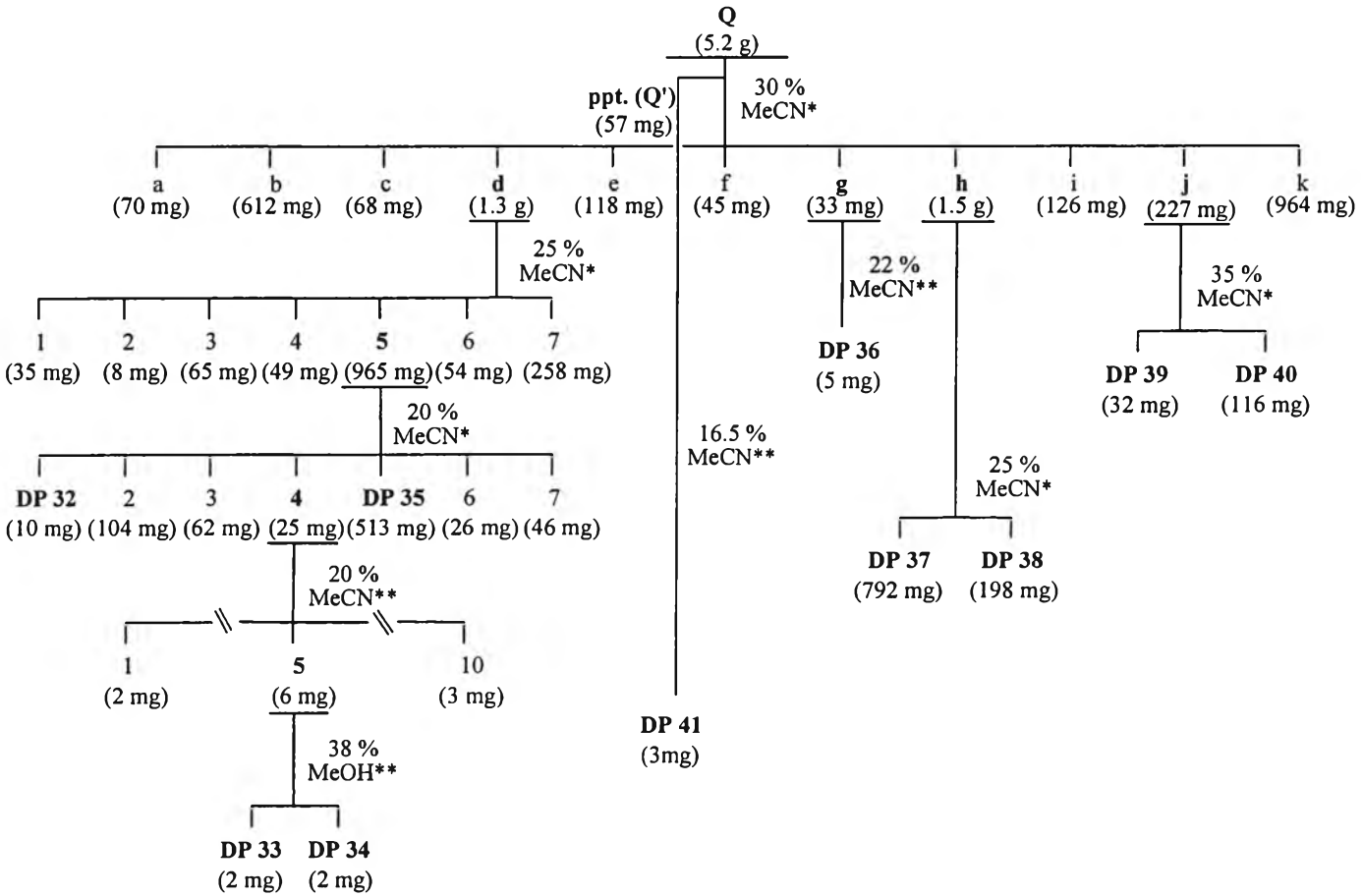
**Scheme 5** Separation of the methanol extract of the heartwood of *Dalbergia parviflora*




* Isolation overed HPLC preparative column: Developsil-Lop-ODS (5x100 cm), Solvent: 25-35% $CH_3CN$

** Isolation overed HPLC preparative column: Capcell-pak ODS (2X25 cm), Solvent: 35% $CH_3CN$

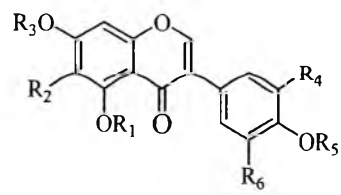
**Scheme 6** Separation of fraction N from the methanol extract of the heartwood of *Dalbergia parviflora*



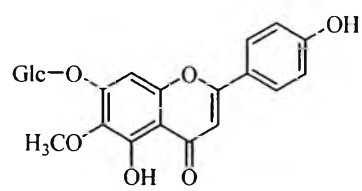

* Isolation overed HPLC preparative column: Developsil-Lop-ODS (5x100 cm), Solvent: 20-35% $CH_3CN$

** Isolation overed HPLC preparative column: Capcell-pak ODS (2X25 cm), Solvent: 16.5-22% $CH_3CN$, 38% $CH_3OH$

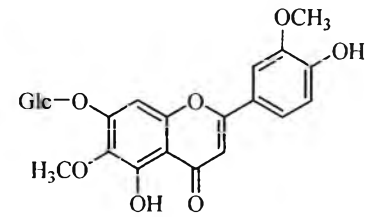
**Scheme 7** Separation of fraction Q from the methanol extract of the heartwood of *Dalbergia parviflora*



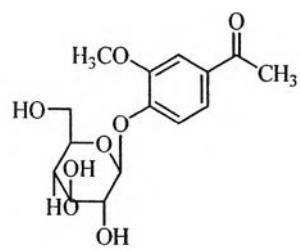
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|---|
| BC1 **[14]** | H | $OCH_3$ | H | H | H | H |
| BC2 **[7]** | $CH_3$ | $-O-CH_2-$ | | $OCH_3$ | $CH_3$ | $OCH_3$ |
| BC3 **[5]** | H | $OCH_3$ | H | OH | $CH_3$ | $OCH_3$ |
| BC4 **[412]** | H | $OCH_3$ | H | OH | H | H |
| BC5 **[13]** | H | $OCH_3$ | Glc | H | H | H |
| BC6 **[413]** | H | $OCH_3$ | Glc | $OCH_3$ | H | H |
| BC7 **[414]** | H | $OCH_3$ | Glc | OH | $CH_3$ | H |
| BC8 **[4]** | H | $OCH_3$ | Glc | OH | $CH_3$ | $OCH_3$ |



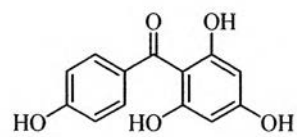
BC9 **[415]**



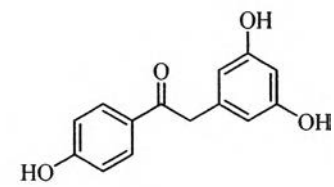
BC10 **[416]**



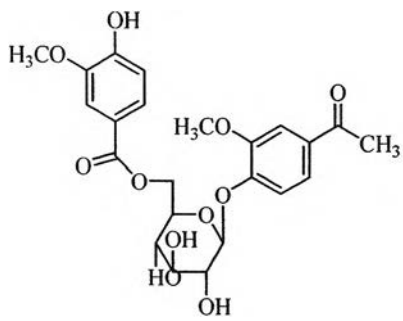
BC11 **[417]**



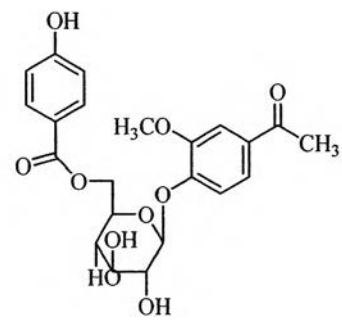
BC12 **[418]**



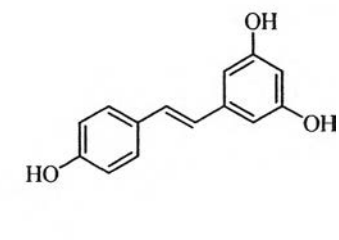
BC13 **[419]**



BC14 **[420]**

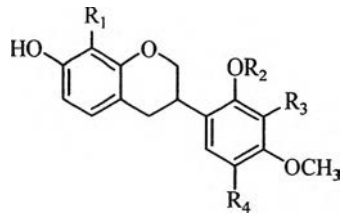


BC15 **[421]**

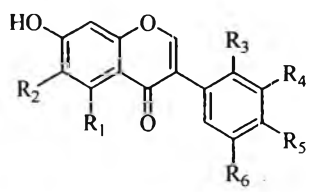


BC16 **[57]**

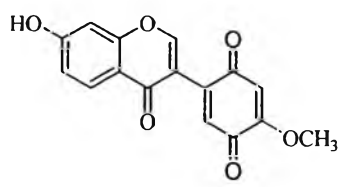
**Figure 3** Structure of compounds isolated from the rhizomes of *B. chinensis*



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| DP1 [422] | $OCH_3$ | H | OH | H |
| DP2 [77] | H | $CH_3$ | OH | H |
| DP13 [427] | OH | $CH_3$ | OH | H |
| DP18 [431] | H | H | OH | H |
| DP25 [78] | H | H | H | H |
| DP30 [183] | H | H | H | $OCH_3$ |

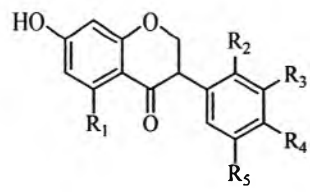


| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|---|
| DP3 [423] | OH | H | $OCH_3$ | H | $OCH_3$ | $OCH_3$ |
| DP7 [424] | OH | H | $OCH_3$ | H | $-OCH_2O-$ | |
| DP9 [95] | OH | H | H | H | $OCH_3$ | H |
| DP11 [425] | OH | H | $OCH_3$ | H | $OCH_3$ | H |
| DP19 [14] | OH | $OCH_3$ | H | H | OH | H |
| DP20 [432] | OH | H | $OCH_3$ | OH | $OCH_3$ | H |
| DP22 [433] | OH | H | H | OH | $OCH_3$ | H |
| DP23 [434] | H | H | $OCH_3$ | H | $OCH_3$ | H |
| DP24 [72] | H | H | H | H | $OCH_3$ | H |
| DP28 [437] | OH | H | OH | H | $OCH_3$ | OH |
| DP31 [439] | H | H | OH | H | $OCH_3$ | OH |
| DP33 [441] | H | H | $OCH_3$ | OH | $OCH_3$ | H |
| DP34 [196] | H | H | H | $OCH_3$ | OH | H |
| DP35 [81] | H | H | H | OH | $OCH_3$ | H |
| DP36 [442] | OH | H | $OCH_3$ | H | OH | H |
| DP38 [3] | OH | H | H | H | OH | H |

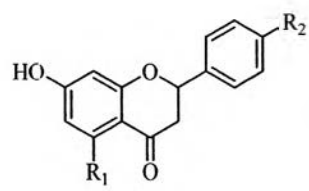


DP41 [443]

**Figure 4** Structure of compounds isolated from the heartwood of *D. parviflora*

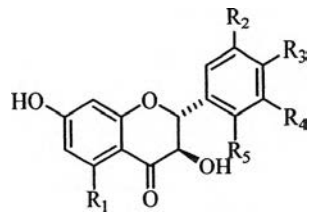



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| DP4 **[197]** | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H |
| DP5 **[252]** | H | $OCH_3$ | H | $-OCH_2O-$ | |
| DP6 **[175]** | H | $OCH_3$ | H | $OCH_3$ | H |
| DP15 **[429]** | OH | $OCH_3$ | OH | $OCH_3$ | H |
| DP16 **[430]** | H | H | OH | $OCH_3$ | H |
| DP17 **[177]** | H | $OCH_3$ | OH | $OCH_3$ | H |
| DP21 **[176]** | H | OH | H | $OCH_3$ | H |
| DP29 **[438]** | H | $OCH_3$ | H | $OCH_3$ | OH |

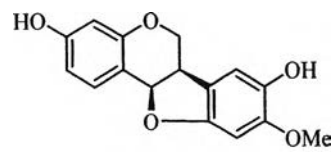



| | $R_1$ | $R_2$ |
|---|---|---|
| DP8 **[174]** | OH | H |
| DP37 **[251]** | OH | OH |
| DP39 **[84]** | H | OH |

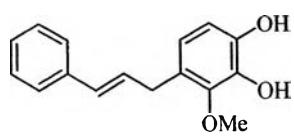
**Figure 4** Structure of compounds isolated from the heartwood of *D. parviflora* (continued)



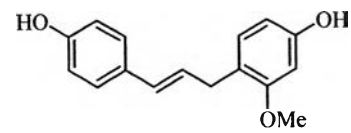
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| DP14 **[428]** | OH | H | H | H | H |
| DP27 **[436]** | H | $OCH_3$ | OH | H | H |
| DP32 **[440]** | H | H | $OCH_3$ | OH | OH |



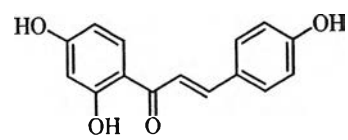
DP12 **[426]**



DP10 **[355]**



DP26 **[435]**



DP40 **[83]**

**Figure 4** Structure of compounds isolated from the heartwood of *D. parviflora* (continued)

## 6. Physical and Spectra data of Isolated Compounds

### 6.1 Compound BC1 (Tectorigenin)

Compound BC1 was obtained as amorphous powder (90 mg; $9\times10^{-3}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 301 (calcd for $C_{16}H_{13}O_6$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 20, Table 6

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO-$d_6$, Table 6

### 6.2 Compound BC2 (Irisflorentin)

Compound BC2 was obtained as amorphous powder (59 mg; $5.9\times10^{-3}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 387 (calcd for $C_{20}H_{19}O_8$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 21, Table 7

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO-$d_6$; Figure 22, Table 7

### 6.3 Compound BC3 (Irigenin)

Compound BC3 was obtained as amorphous powder (115 mg; $1.15\times10^{-2}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 361 (calcd for $C_{18}H_{17}O_8$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 23, Table 8

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO-$d_6$; Figure 24, Table 8

### 6.4 Compound BC4 (Irilin D)

Compound BC4 was obtained as amorphous powder (4 mg; $4\times10^{-4}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 317 (calcd for $C_{16}H_{13}O_7$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 25, Table 9

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO-$d_6$; Figure 26, Table 9

### 6.5 Compound BC5 (Tectoridin)

Compound BC5 was obtained as amorphous powder (96.1 mg; $9.6\times10^{-3}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 463 (calcd for $C_{22}H_{23}O_{11}$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 27, Table 10

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Table 10

**6.6 Compound BC6 (Iristectorin B)**

Compound BC6 was obtained as amorphous powder (155.5 mg; $1.5\times10^{-2}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 493 (calcd for $C_{23}H_{25}O_{12}$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 28, Table 11

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 29, Table 11

**6.7 Compound BC7 (Iristectorin A)**

Compound BC7 was obtained as amorphous powder (30 mg; $3\times10^{-3}$ % base on dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 493 (calcd for $C_{23}H_{25}O_{12}$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 33, Table 12

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO-$d_6$; Figure 34, Table 12

**6.8 Compound BC8 (Iridin)**

Compound BC8 was obtained as amorphous powder (283.3 mg; $2.8\times10^{-2}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 523 (calcd for $C_{24}H_{27}O_{13}$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 38, Table 13

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 39, Table 13

**6.9 Compound BC9 (Hispiduloside)**

Compound BC 9 was obtained as amorphous powder (20.9 mg; $2.1\times10^{-3}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 463 (calcd for $C_{22}H_{23}O_{11}$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 40, Table 14

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 41, Table 14

**6.10 Compound BC10 (Jaceoside)**

Compound BC10 was obtained as amorphous powder (11.2 mg; $1.1\times10^{-3}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 493 (calcd for $C_{23}H_{25}O_{12}$)

$^{1}$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 42, Table 15

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Table 15

### 6.11 Compound BC11 (Androsin)

Compound BC11 was obtained as amorphous powder (9.7 mg; $9.7\times10^{-4}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 329 (calcd for $C_{15}H_{21}O_8$)

$^{1}$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 47, Table 16

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 49, Table 16

### 6.12 Compound BC12 (Iriflophenone)

Compound BC12 was obtained as amorphous powder (3.9 mg; $3.9\times10^{-4}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 247 (calcd for $C_{13}H_{11}O_5$), Figure 50

$^{1}$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 51, Table 17

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 52, Table 17

### 6.13 Compound BC13 (Belamphenone)

Compound BC13 was obtained as amorphous powder (3.6 mg; $3.6\times10^{-4}$ % of dried weight of rhizomes)

HRFABMS : $[M+H]^+$ *m/z* 245.0802 (calcd for $C_{14}H_{13}O_4$, 245.0814), Figure 51

UV : $\lambda_{max}$ nm (log ε), in MeOH
219 (sh) (4.00), 278 (3.87) nm

$^{1}$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 56, Table 18

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 57, Table 18

### 6.14 Compound BC14 (Belalloside A)

Compound BC14 was obtained as amorphous powder (1.5 mg; $1.5\times10^{-4}$ % of dried weight of rhizomes)

HRFABMS : $[M+Na]^+$ *m/z* 501.1357 (calcd For $C_{23}H_{26}O_{11}Na$, 501.1373)

$[\alpha]_D^{25}$ : -37.7 °( *c* 0.2, MeOH)

UV : $\lambda_{max}$ nm (log ε), in MeOH
221 (sh) (4.27), 264 (3.78), 294 (3.90) nm

$^{1}$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 61, Table 19

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 62, Table 19

### 6.15 Compound BC15 (Belalloside B)

Compound BC15 was obtained as amorphous powder (1.5 mg; $1.5\times10^{-4}$ % base on dried weight of rhizomes)

HRFABMS : $[M+Na]^+$ *m/z* 471.1247 (calcd for $C_{22}H_{24}O_{10}Na$, 471.1267)

$[\alpha]_D^{25}$ : + 9.5° (*c* 0.2, MeOH)

UV : $\lambda_{max}$ nm (log ε), in MeOH; Figure
224 (sh) (414), 260 (3.86), 299 (3.64) nm

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 66, Table 20

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 67, Table 20

### 6.16 Compound BC16 (Resveratrol)

Compound BC16 was obtained as amorphous powder (385 mg; $3.8\times10^{-2}$ % of dried weight of rhizomes)

FABMS : $[M+H]^+$ *m/z* 229 (calcd for $C_{14}H_{13}O_3$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 68, Table 21

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 69, Table 21

### 6.17 Compound DP1 ((±)-Khriol A)

Compound DP1 was obtained as amorphous powder (140 mg; $4.25\times10^{-2}$ % of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 318.1115 (calcd for $C_{17}H_{18}O_6$, 318.1103 ); Figure 66

UV : $\lambda_{max}$ nm (log ε) in MeOH
277 (3.68), 230 (sh) (4.28) nm

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 71, Table 22

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 73, Table 22

### 6.18 Compound DP2 ((3*R*)-(+)-Mucronulatol)

Compound DP 2 was obtained as amorphous powder (870 mg; 0.26 % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 302 (calcd for $C_{17}O_{18}H_5$ ); Figure 76

$[\alpha]_D^{25}$ : + 19.3 ° (*c* 1.0 MeOH )

CD : [$\theta$] (nm) 3700 (283), -14600 (230), 27500 (211)(c $2.2\times10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 77, Table 23

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 79, Table 23

### 6.19 Compound DP3 (7-Demethoxybustigenin)

Compound DP3 was obtained as amorphous (8 mg; $2.43 \times 10^{-3}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 344 (calcd for $C_{18}H_{16}O_7$ ); Figure 83

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone- $d_6$; Figure 84, Table 24

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone- $d_6$; Figure 86, Table 24

### 6.20 Compound DP4 ((±)- 3′- Methoxyviolanone)

Compound DP4 was obtained as amorphous powder (3.8 g; 1.15 % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 331 (calcd for $C_{18}H_{19}O_6$ ) ; Figure 87

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone- $d_6$; Figure 88, Table 25

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone- $d_6$; Figure 89, Table 25

### 6.21 Compound DP5 ((±)- Onogenin)

Compound DP5 was obtained as amorphous powder (130 mg; $3.94 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 314 (calcd for $C_{17}H_{14}O_6$ ); Figure 93

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone- $d_6$; Figure 94, Table 26

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone- $d_6$; Figure 95, Table 26

### 6.22 Compound DP6 ((3*R*)-(+)- Sativanone)

Compound DP6 was obtained as amorphous powder (310 mg; $9.4 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 301 (calcd for $C_{17}H_{17}O_5$ ); Figure 96

$[\alpha]_D^{25}$ : + 9.7 ° (*c* 1.0, MeOH)

CD : $[\theta]$ (nm) 5200 (280), -12500 (235) (*c* $1.9 \times 10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 97, Table 27

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 98, Table 27

### 6.23 Compound DP7 (Khrinone D)

Compound DP7 was obtained as amorphous powder (5 mg; $1.52 \times 10^{-3}$ % of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 328.0557 (calcd for $C_{17}H_{12}O_7$, 328.0583); Figure 102

UV : $\lambda_{max}$ nm (log ε) in MeOH

299.5 (4.02), 260 (4.26), 225 (sh) (4.34)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone- $d_6$; Figure 103, Table 28

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone- $d_6$; Figure 105, Table 28

### 6.24 Compound DP8 ((2*S*)- (-)- Pinocembrin)

Compound DP8 was obtained as amorphous powder (85 mg; $2.58 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 257(calcd for $C_{15}H_{13}O_4$); Figure 106

$[\alpha]_D^{25}$ : -48.3 ° (*c* 1.0, MeOH)

CD : $[\theta]$ (nm) -49300 (283), 69300 (216) (*c* $2.6 \times 10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 107, Table 29

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 108, Table 29

### 6.25 Compound DP9 (Biochanin A)

Compound DP9 was obtained as amorphous powder (320 mg; $9.71 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 285(calcd for $C_{16}H_{13}O_5$); Figure 112

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 113, Table 30

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 115, Table 30

### 6.26 Compound DP10 (Hydroxyobtustyrene)

Compound DP10 was obtained as amorphous powder (55 mg; $1.67 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 256(calcd for $C_{16}H_{16}O_3$); Figure 116

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone- $d_6$; Figure 117, Table 31

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone- $d_6$; Figure 119, Table 31

**6.27 Compound DP11 (2′ - Methoxybiochanin A)**

Compound DP11 was obtained as amorphous powder (45 mg; 1.36 × $10^{-2}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 315(calcd for $C_{17}H_{15}O_6$); Figure 120

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone- $d_6$; Figure 121, Table 32

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone- $d_6$; Figure 123, Table 32

**6.28 Compound DP12 ((6a*R*, 11a*R*) - (-) - 3, 8 – dihydroxy – 9 - methoxy pterocarpan)**

Compound DP12 was obtained as amorphous powder (36 mg; 1.09 × $10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 286(calcd for $C_{16}H_{14}O_5$)

$[\alpha]_D^{25}$ : -174.4 ° (*c* 0.5, MeOH)

CD : $[\theta]$ (nm) 12200 (296), -73800 (234) (*c* 2.5 × $10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$, Table 33

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$, Table 33

**6.29 Compound DP13 ((3*S*)-(+)-8 - Demethylduartin)**

Compound DP13 was obtained as amorphous powder (113 mg; 3.43 × $10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 318(calcd for $C_{17}H_{18}O_6$)

$[\alpha]_D^{25}$ : +16.3 ° (*c* 1.0 , MeOH)

CD : $[\theta]$ (nm) 6400 (238), -17700 (215) (*c* 2.5 × $10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$, Table 34

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$, Table 34

**6.30 Compound DP14 ((2*R*, 3*R*)-(+)- Pinobanksin)**

Compound DP14 was obtained as amorphous powder (800 mg; 0.243 % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 273(calcd for $C_{15}H_{13}O_5$)

$[\alpha]_D^{25}$ : +12.4 ° (*c* 1.0, MeOH)

CD : $[\theta]$ (nm) -37800 (288), 71000 (222) (*c* 2.1 × $10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$, Table 35

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$, Table 35

### 6.31 Compound DP15 ((3*S*) - (-)- Secundiflorol H)

Compound DP15 was obtained as amorphous powder (2.3 g; 0.7 % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 333(calcd for $C_{17}H_{17}O_7$)

$[\alpha]_D^{25}$ : -7.9 ° (*c* 1.0, MeOH)

CD : $[\theta]$ (nm) -8900 (282), 8900 (221) (*c* $1.8 \times 10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$, Table 36

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$, Table 36

### 6.32 Compound DP16 ((±)- 7, 3′ - Dihydroxy - 4′ - methoxyisoflavanone)

Compound DP16 was obtained as amorphous powder (125 mg; $3.79 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 287(calcd for $C_{16}H_{15}O_5$); Figure 124

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 125, Table 37

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 127, Table 37

### 6.33 Compound DP17 ((±)- Violanone)

Compound DP17 was obtained as amorphous powder (3.17g; 0.96% of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 317(calcd for $C_{17}H_{17}O_6$); Figure 128

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 129, Table 38

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 131, Table 38

### 6.34 Compound DP18 ((3*R*)- (+) – Arizonicanol A)

Compound DP18 was obtained as amorphous powder (45 mg; $1.36 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 289(calcd for $C_{16}H_{17}O_5$); Figure 135

$[\alpha]_D^{25}$ : +4.1 ° (*c* 0.2, MeOH)

CD : $[\theta]$ (nm) 1900 (280), -8600 (232) (*c* $4.18 \times 10^{-5}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 136, Table 39

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 137, Table 39

**6.35 Compound DP19 (Tectorigenin)**

Compound DP19 was obtained as amorphous powder (610 mg; 0.19 % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 301(calcd for $C_{16}H_{13}O_6$); Figure 138

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 139, Table 40

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 140, Table 40

**6.36 Compound DP20 (Khrinone C)**

Compound DP20 was obtained as amorphous powder (150 mg; $4.55\times10^{-2}$ % of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 330.0746 (calcd for $C_{17}H_{14}O_7$, 330.0740); Figure 141

UV : $\lambda_{max}$ nm (log ε) in MeOH

330 (3.68), 292 (4.04), 259(sh) (4.53)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 142, Table 41

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 144, Table 41

**6.37 Compound DP21 ((±) - Vestitone)**

Compound DP21 was obtained as amorphous powder (25 mg; $7.58\times10^{-3}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 287(calcd for $C_{16}H_{15}O_5$); Figure 147

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 148, Table 42

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 149, Table 42

**6.38 Compound DP22 (Pratensein)**

Compound DP22 was obtained as amorphous powder (715 mg; 0.22% of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 301(calcd for $C_{16}H_{13}O_6$); Figure 150

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 151, Table 43

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 153, Table 43

**6.39 Compound DP23 (2′ - Methoxyformononetin)**

Compound DP23 was obtained as amorphous powder (10 mg; $3.03\times10^{-3}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 299(calcd for $C_{17}H_{15}O_5$); Figure 154

$^1H$ NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 155, Table 44

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 157, Table 44

**6.40 Compound DP24 (Formononetin)**

Compound DP24 was obtained as amorphous powder (30 mg; $9.1\times10^{-3}$ % of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 269(calcd for $C_{16}H_{13}O_4$)

$^1H$ NMR : δ ppm, 400 MHz, in acetone-$d_6$; Figure 158, Table 45

$^{13}C$ NMR : δ ppm, 100.4 MHz, in acetone-$d_6$, Table 45

**6.41 Compound DP25 ((3*R*)-(-) - Vestitol)**

Compound DP25 was obtained as amorphous powder 150 mg; $4.55 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 272(calcd for $C_{16}H_{16}O_4$)

$[\alpha]_D^{25}$ : -11.2 ° (*c* 0.6, MeOH)

CD :[$\theta$] (nm) 3700 (288), -9700 (233), - 10100 (216)(*c* $4.35 \times 10^{-5}$, MeOH)

$^1H$ NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 160, Table 46

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 162, Table 46

**6.42 Compound DP26 (Xenognosin)**

Compound DP26 was obtained as amorphous powder (15 mg; $4.55\times10^{-3}$ % base on dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 256(calcd for $C_{16}H_{16}O_3$); Figure 163

$^1H$ NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 164, Table 47

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 166, Table 47

**6.43 Compound DP27 ((2*R*, 3*R*) - Dalparvinol A)**

Compound DP27 was obtained as amorphous powder 22 mg; $6.67 \times 10^{-3}$ % of dried weight of heartwood)

HRFABMS : $[M+H]^+$ *m/z* 303.0846 (calcd for $C_{16}H_{15}O_6$, 303.0824)

$[\alpha]_D^{25}$ : -5.0 ° (*c* 0.3, MeOH)

CD : [$\theta$] (nm) -18100 (305), -4760 (270), 10463 (239), 29500(215) (*c* 2.1 ×

$10^{-4}$, MeOH)

UV : $\lambda_{max}$ nm (log ε) in MeOH

311 (3.86), 277 (4.12), 233(4.25), 215 (sh) (4.45)

$^1$H NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$, Table 48

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$, Table 48

### 6.44 Compound DP28 (Khrinone B)

Compound DP28 was obtained as amorphous powder 980 mg; 0.3% of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 316.0609 (calcd for $C_{16}H_{12}O_7$, 316.0583); Figure 170

UV : $\lambda_{max}$ nm (log ε) in MeOH

298 (4.21), 260 (4.49)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 171, Table 49

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 173, Table 49

### 6.45 Compound DP29 ((±)-Dalparvin)

Compound DP29 was obtained as amorphous powder 520 mg; 0.16% of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 316.0947 (calcd for $C_{17}H_{16}O_6$, 316.0947)

UV : $\lambda_{max}$ nm (log ε) in MeOH

312(398), 278 (4.19), 230(4.26), 215 (sh) (4.46)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 174, Table 50

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$; Figure 176, Table 50

### 6.46 Compound DP30 ((3*R*)-(-) - 5′-Methoxyvestitol)

Compound DP30 was obtained as amorphous powder 140 mg; $4.25 \times 10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 302 (calcd for $C_{17}H_{18}O_5$)

$[\alpha]_D^{25}$ : -10.0 ° (*c* 0.2, MeOH)

CD : [$\theta$] (nm) -7500 (237), (*c* $1.87 \times 10^{-4}$, MeOH)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$, Table 51

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in acetone-$d_6$, Table 51

### 6.47 Compound DP31 (Khrinone A)

Compound DP31was obtained as amorphous powder 406 mg; 0.12% of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 300.0647 (calcd for $C_{16}H_{12}O_6$, 300.0634); Figure 173

UV : $\lambda_{max}$ nm (log ε) in MeOH
300(4.37), 264 (4.32), 248(4.43)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO- $d_6$; Figure 178, Table 52

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO- $d_6$; Figure 180, Table 52

### 6.48 Compound DP32 ((2*R*, 3*R*)-(-)-Dalparvinol B)

Compound DP32 was obtained as amorphous powder 6 mg; $1.82\times10^{-3}$ % of dried weight of heartwood)

HRFABMS : $[M]^+$ *m/z* 318.0755 (calcd for $C_{16}H_{14}O_7$, 318.0740)

$[\alpha]_D^{25}$ : -407.8 ° (*c* 0.7, MeOH)

CD : $[\theta]$ (nm) -10900 (276), 21900 (315), (*c* $2.19\times10^{-3}$, MeOH)

UV : $\lambda_{max}$ nm (log ε) in MeOH
310 (3.91), 278 (4.11), 231(sh), 215 (sh) (4.21)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 183, Table 53

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 185, Table 53

### 6.49 Compound DP33 (Krinone E)

Compound DP33 was obtained as amorphous powder (2 mg; $6.07\times10^{-4}$ % of dried weight of heartwood)

HRFABMS : $[M+H]^+$ *m/z* 315.0872 (calcd for $C_{17}H_{15}O_6$, 315.0869)

FABMS : $[M]^+$ *m/z* 31 (calcd for $C_{17}H_{14}O_6$)

UV : $\lambda_{max}$ nm (log ε) in MeOH
298(4.04), 248 (4.38), 239(sh) (4.40)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 189, Table 54

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 190, Table 54

### 6.50 Compound DP34 (3′ - Methoxydaidzein)

Compound DP34 was obtained as amorphous powder (2 mg; $6.07\times10^{-4}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 284 (calcd for $C_{16}H_{12}O_5$)

$^1H$ NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$; Figure 193, Table 55

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 195, Table 55

### 6.51 Compound DP35 (Calycosin)

Compound DP35 was obtained as amorphous powder (513 mg; 0.16% of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 284 (calcd for $C_{16}H_{12}O_5$)

$^1H$ NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$; Figure 196, Table 56

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 197, Table 56

### 6.52 Compound DP36 (Theralin)

Compound DP36 was obtained as amorphous powder (5 mg; $1.52\times10^{-3}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 300 (calcd for $C_{16}H_{12}O_6$)

$^1H$ NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$; Figure 201, Table 57

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 203, Table 57

### 6.53 Compound DP37 ((2*S*)-(-) - Naringenin)

Compound DP37 was obtained as amorphous powder 792 mg; 0.24% of dried weight of heartwood)

FABMS : $[M+H]^+$ *m/z* 273(calcd for $C_{15}H_{13}O_5$)

$[\alpha]_D^{25}$ : -79.5 ° (*c* 1.0 , MeOH)

CD : $[\theta]$ (nm) -23300 (287), - 7500 (235) (*c* $2.4 \times 10^{-4}$, MeOH)

$^1H$ NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 204, Table 58

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 205, Table 58

### 6.54 Compound DP38 (Genistein)

Compound DP38 was obtained as amorphous powder (198 mg; $6.0\times10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 270 (calcd for $C_{15}H_{10}O_5$)

$^1H$ NMR : $\delta$ ppm, 400 MHz, in MeOH-$d_4$; Figure 206, Table 59

$^{13}C$ NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 207, Table 59

### 6.55 Compound DP39 ((2*S*)-(-) - Liquiritigenin)

Compound DP39 was obtained as amorphous powder (32 mg; $9.7\times10^{-3}$ % of dried weight of heartwood)

$[\alpha]_D^{25}$ : -14.5 ° (*c* 0.1, MeOH)

FABMS : $[M]^+$ *m/z* 256 (calcd for $C_{15}H_{12}O_4$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 208, Table 60

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 209, Table 60

### 6.56 Compound DP40 (Isoliquiritigenin)

Compound DP40 was obtained as amorphous powder (116 mg; $3.52\times10^{-2}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 256 (calcd for $C_{15}H_{12}O_4$)

$^1$H NMR : $\delta$ ppm, 400 MHz, in acetone-$d_6$; Figure 210, Table 61

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in MeOH-$d_4$; Figure 211, Table 61

### 6.57 Compound DP41 (Bowdichione)

Compound DP41 was obtained as amorphous powder 3 mg; $9.1\times10^{-4}$ % of dried weight of heartwood)

FABMS : $[M]^+$ *m/z* 298 (calcd for $C_{16}H_{10}O_6$)

UV : $\lambda_{max}$ nm (log ε) in MeOH

297(4.07), 249 (4.19)

$^1$H NMR : $\delta$ ppm, 400 MHz, in DMSO-$d_6$; Figure 212, Table 62

$^{13}$C NMR : $\delta$ ppm, 100.4 MHz, in DMSO-$d_6$; Table 62

## 7. Evaluation for Estrogenic Activities.

In this study, the estrogenic activities are evaluated by monitoring the proliferation of cells and stimulatory potency of the substances in estrogen receptors containing cell lines, MCF-7 and T47D (Soto *et al.*, 1995). In order to confirm the estrogenic activity of potential compounds, the luciferase transient assay was performed. This luciferase reporter gene assay was conducted according to the procedure of Edmunds and co-workers in 1997 with minor modifications.

### 7.1 Cell Culture

Human breast cancer cells, MCF-7 and T47D were purchased from the American Type Culture Collection (Manassas, VA). The MCF-7 cells were grown in MEM supplemented with 6 ng/mL insulin, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, 10% FBS, and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) under a 5% $CO_2$ humidified atmosphere at 37 °C. The T47D cells were grown in RPMI-1640 supplemented with 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, 10% FBS, and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) under a 5% $CO_2$ humidified atmosphere at 37°C.

### 7.2 Cell Proliferation.

Cells were seeded into 96-well tissue culture plates in 5% DCC treated, FBS supplemented RPMI phenol red-free medium at a density of $1x10^4$ cells/well. Test compounds were added in 70% EtOH solution (control contained 0.7 % ethanol) and incubated at 37°C with 5% $CO_2$ for 96 h. In all experiments, serial dilutions of estradiol were added as positive control. To evaluate relative cell concentrations, Alamar Blue reagent was used. After 3h, fluorescence was measured at 590 nm with excitation at 530 nm using a FL500 spectrophotometer (BIO-TEK Instruments Inc, USA).

### 7.3 Construction of Luciferase Reporter Plasmid

The reporter gene EREx3-pGV-P2 was constructed using luciferase reporter gene pGV-p2 basis vector (Toyo Ink Co. Tokyo). Three tandem repeats of the consensus ERE oligonucleotide (GCTAGCAGGTCACAGTGACCT) upstream of the human TATA promoter sequence were inserted into the *Nhe* I-*Xho* I site of the multiple cloning site of the pGV-p2 basis vector.

### 7.4 Luciferase Reporter Gene Assay

The luciferase reporter gene assay was conducted according to the procedure of Edmunds and co-worker with minor modifications. MCF-7 or T47D cells were placed in phenol red-free MEM supplemented with 5% DCC FBS for 48h prior to plating. The cells were transfected for 12h in OPTI-MEM with 2 μg of

EREx3-pGVP2 plasmid (containing three copies of the ERE linked to the luciferase gene) using LIPOFECTAMINE in a 12:1 ratio (24 μL of liquid/2 μg of DNA) according to the manufacturer's instruction. The medium was changed for phenol red-free MEM with 5% DCC-FBS, and the cells were incubated for 12h. After 12h, luciferase transfected-MCF-7 (MCF-7/Luc) or luciferase transfected-T47D (T47D/Luc) cells ($2x10^4$/well) were seeded in 96 well plates with the same medium (90 μL), and after culturing for 24h, a test compound dissolved in the medium (10 μL) was added to each well, and further incubated for 12h. In all experiments, serial dilutions of estradiol were added as positive control. After treatment with test compounds, the cells were washed twice with PBS and lysed by treatment with 20 μL lysis buffer (Pica gene assay kit; Toyo Ink Co. Tokyo) per well for 10 min at room temperature. Luciferase activities for the cell extracts were determined using Luciferase Substrate (Pica gene assay kit; Toyo Ink Co. Tokyo) in a luminometer (Labsystems Luminoskan RS).

### 7.5 Preparation of Herb Extracts.

Herbs were extracted with methanol by refluxing for 1 h., and the insoluble contents were filtered off at room temperature. The methanolic solution was concentrated under vacuum. For assays involving methanolic extracts and phytochemicals, serial dilutions of the samples to be tested was made in a 70% aqueous ethanol solution at concentration 100-fold higher than the desired final concentrations. The 70% ethanolic solutions were then diluted 10-fold with cell culture medium. When tested for estrogenic activity, the final concentration of the organic solvent never exceeded 1%. This concentration was shown not to interfere with the different assays used, as a result of control experiments.

### 7.6 Data and Statistical Analysis.

Statistical differences were determined by analysis of variance followed by Dunnett's multiple comparison tests. Statistical significance was established at the $p<0.05$ level.

### 7.7 Calculation of Eq $E_{10}$ and Eq $E_{100}$.

$EqE_{10}$ and $EqE_{100}$ represent the concentration of the compound that stimulated the cell proliferations equivalent to 10 and 100 pM estradiol, respectively. These values were determined by linear regression analysis, which 3-5 different concentrations in quadruplicate versus ratio of cell proliferation to control were plotted.