

## Isolation and Evaluation of Antimitotic Activity of Phenolic Compounds from *Pouteria campechiana* Baehni.

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**This study was undertaken to determine the compound responsible for the antimitotic activity of ethyl acetate extracts obtained from the leaves of *Pouteria campechiana* Baehni. Six stilbenes and six flavonoid glycosides were purified and identified after chromatographic separation and spectroscopic analysis. The distilbene ampelopsin B was found to arrest mitosis in a cell-based primary screen to monitor cell-cycle progression and was also determined to be a weak microtubule depolymerizer in a secondary assay.**

Key Words: Stilbenes, ampelopsin B, flavonoid glycosides, Sapotaceae

### INTRODUCTION

*Pouteria campechiana* is a member of the family Sapotaceae and it is cultivated in the Philippines for its edible fruit. This is the first report on the isolation of stilbenoids from this family. Stilbenoids have been established to be present in the families Dipterocarpaceae (Tanaka et al. 2001; Ito et al. 2003; Tanaka et al. 2000a), Leguminosae (Luo et al. 2001; Ohyama et al 1994a; Ohyama et al 1994b), and Vitaceae (Yan et al 2002; Li et al. 1998; Ito et al. 1999; Dai et al. 1998). This group of compounds exhibits a wide range of biological activity including anti- HIV (Kim et al. 2003), anti-tumor (Liu et al. 2004), anti-inflammatory (Zgoda-Pols et al. 2002), antimicrobial (Oshima et al. 1995a), and hepatoprotective action (Oshima et al. 1990).

The ethyl acetate fraction of *Pouteria campechiana* Baehni is part of the Philippine plant extract library deposited at the Institute of Chemistry and Cell Biology,

Harvard Medical School for high-throughput screening. This plant extract was selected for research studies following a positive result in a cell-based assay for anti-mitotic activity using HeLa cells. Additionally, no phytochemical and bioactivity studies have been reported in this species to date.

### MATERIALS AND METHODS

#### General Methods

<sup>1</sup>H NMR spectra were obtained at 500 MHz and <sup>13</sup>C NMR spectra were run at 125 MHz using Varian Oxford NMR AS500 spectrometer. COSY, HMQC, HMBC, and NOESY were obtained using standard Varian pulse sequences. LCMS data were obtained using a Micromass Platform LC-Z spectrometer, equipped with a Waters 2690 LC system and Waters Photodiode array detector, and processed using MassLynx software (Waters Corporation, Milford, MA, www.waters.com). HPLC analyses were

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performed on an Agilent 1100 system equipped with diode array detector and operated with Chemstation software (Agilent Technologies). C-18 Discovery semi-prep column was used with solvent A=H<sub>2</sub>O, 100% and B=MeCN, 0% at t=0 min and A=H<sub>2</sub>O, 0% and B=MeCN, 100% at t=25 min and a flow rate of 4 ml/min.

### Chemicals

The methanol used for soaking the sample was technical grade and distilled before use. All the solvents used for extraction, isolation, and purification were HPLC grade. Column chromatography was carried out on a silica gel 60 (Fisher Chemicals, 230-400 mesh) and Sephadex LH-20 (Aldrich). HPLC separations were done using a Zorbax Eclipse Semi-Prep column and Discovery HS-C18 column (Supelco, 250 x 101.86nm, 5mm particle size) with a 4mL/min flow rate.

### Plant Material

*P. campechiana* leaves were collected from Paniqui, Tarlac, Philippines in March 2003. The sample was authenticated by Dr. Danilo Lagunza, taxonomist from the Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman and a voucher specimen with accession no. 14513 was deposited.

### Extraction and Isolation

The dried leaves (2.3 kg) of *P. campechiana* were soaked in MeOH overnight at room temperature and concentrated in vacuo. Water was added to the methanolic concentrate to make 90% solution and defatted with hexane. Additional water was added to the alcoholic fraction to make 60% methanolic fraction, then partitioned with dichloromethane followed by ethyl acetate. About 7g of the ethyl acetate extract was subjected to silica gel chromatography and eluted with increasing polarity of hexane – ethyl acetate. Ten fractions were pooled based on their TLC profile. Fractions 2-5 were further purified using Sephadex LH-20 column and eluted with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:1) to give (1) (5mg), (2) (90 mg), (3) (9 mg), (4) (40 mg), (5) (99 mg), (6) (281 mg), and (7) (52 mg). Fraction 7 was subjected to semi-prep HPLC to give (8) (8 mg) at  $t_R$ =10.7 min, (9) (30 mg) at  $t_R$ =12.8 min, (10) (26 mg) at  $t_R$ =11.2 min, and (11-12) (15 mg) at  $t_R$ =16.8 min.

### Visual screen assay for mitosis

Compounds 1 – 12 were evaluated for their anti-mitotic activity at the Institute of Chemistry and Cell Biology, Harvard Medical School, using a mammalian cell-based fluorescence imaging assay. HeLa cells were synchronized using a double thymidine block and plated in 384-well plates (8,000 cells per well) using an automated dispenser

(Biotek MicroFill). Compounds 1-12 were added to a final concentration of 50  $\mu$ M (one compound per well). Following a 16 h incubation (37° C, 5% CO<sub>2</sub>) cells were fixed and processed for analysis of epitopes relevant to cell cycle state (actin, DNA (for primary and secondary screens), microtubules (for secondary screen)). Briefly, a “fix and stain” cocktail containing (final concentrations within a well) 4% formaldehyde, 0.5% Triton X-100, 0.1  $\mu$ g/mL TRITC-Phalloidin (to stain actin), and 1  $\mu$ g/mL Hoechst 33342 (to stain DNA) in PBS was incubated with the cells for 30 min at 25° C. Fixative was then removed by aspiration and followed by 2 washes with Tris-buffered saline (TBS) with 0.1% Tween20. To analyze the microtubule cytoskeleton, 2% BSA was included in the “fix and stain” cocktail described above, and the initial incubation with this cocktail was followed by a 30 min incubation with the anti-microtubule antibody, DM1 $\alpha$  (1:250 dilution, Sigma, St Louis, MO), and Alexa488-coupled goat anti-mouse secondary antibody (1:1000 dilution, Molecular Probes, CA). Washes were then performed as described above. In both cases, images of treated wells were acquired using a high throughput automated microscope (e.g. Discovery 1, Molecular Devices, CA) and subsequently analyzed to identify compounds that disrupt cell cycle progression.

(1) UV (MeOH,  $\lambda_{max}$ ) 201nm, 260nm; <sup>1</sup>H-NMR (500MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 3.51, 3.15 (2H, dd, J=18.3, 3.91, H-8a/8a'),  $\delta$ 4.04 (1H, d, J=11.7, H-8b),  $\delta$ 5.13 (1H, t, J=3.91, H-7a),  $\delta$ 5.65 (1H, d, J=11.7, H-7b),  $\delta$ 6.00 (1H, d, J=2.0, H-12a),  $\delta$ 6.08 (1H, d, J=2.0, H-14b),  $\delta$ 6.20 (1H, d, J=2.0, H-14a),  $\delta$ 6.30 (1H, d, J=2.0, H-12b),  $\delta$ 6.59 (2H, d, J=8.8, H-3a/5a),  $\delta$ 6.68 (2H, d, J=8.3, H-3b/5b),  $\delta$ 6.89 (2H, d, J=8.8, H-2a/6a),  $\delta$ 7.02 (2H, d, J=8.3, H-2b/6b); <sup>13</sup>C-NMR (125MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 32.59 (C-8a),  $\delta$ 35.10 (C-7a),  $\delta$ 48.24 (C-8b),  $\delta$ 87.83 (C-7b),  $\delta$ 94.48 (C-12a),  $\delta$ 100.3 (C-12b),  $\delta$ 103.18 (C-14a),  $\delta$ 104.03 (C-14b),  $\delta$ 114.34 (C-3a/5a),  $\delta$ 114.81 (C-3b/5b),  $\delta$ 118.46 (C-10a),  $\delta$ 122 (C-10b),  $\delta$ 127.6 (C-2a/6a),  $\delta$ 128.87 (C-2b/6b),  $\delta$ 130 (C-1b),  $\delta$ 134 (C-1a),  $\delta$ 137.44 (C-9a),  $\delta$ 141 (C-9b),  $\delta$ 155 (C-4a),  $\delta$ 156 (C-11b/13b),  $\delta$ 157 (C-4b),  $\delta$ 159.5 (C-11a),  $\delta$ 161.3 (C-13a); ES-MS (negative ion mode) m/z 454 [M-H]<sup>-</sup>; ES-MS (positive ion mode) m/z 362 (100). These data are in agreement with those found in the literature (Seo et al. 1999).

(2) UV (MeOH) $\lambda_{max}$  235 nm, 282 nm; <sup>1</sup>H-NMR (500MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 4.84 (1H, d, J=2.7, H-7b),  $\delta$ 5.13 (1H, d, J=9.5, H-8a),  $\delta$ 5.37 (1H, d, J=2.7, H-8b),  $\delta$ 5.69 (1H, d, J=9.5, H-7a),  $\delta$ 5.95 (1H, d, J=2.3, H-12b),  $\delta$ 5.99 (1H, d, J=2.3, H-10b),  $\delta$ 6.15 (2H, d, J=2.0, H-12a),  $\delta$ 6.40 (2H, d, J=8.5, H-3b/5b),  $\delta$ 6.68 (2H, d, J=8.5, H-2b/5b),  $\delta$ 6.91 (2H, d, J=8.8, H-3a/5a),  $\delta$ 7.45 (2H, d, J=8.8, H-2a/6a); <sup>13</sup>C-NMR (125MHz, CD<sub>3</sub>OD, ppm)

$\delta$ 50.44 (C-7b),  $\delta$ 52.9 (C-8a),  $\delta$ 73.83 (C-8b),  $\delta$ 94.3 (C-7a),  $\delta$ 95.32 (C-12b),  $\delta$ 102 (C-12a),  $\delta$ 104.93 (C-10b),  $\delta$ 106.67 (C-10a),  $\delta$ 114.36 (C-3b/5b),  $\delta$ 114.36 (C-14b),  $\delta$ 116.17 (C-3a/5a),  $\delta$ 120.93 (C-14a),  $\delta$ 130.15 (C-2A/6A),  $\delta$ 131.62 (C-2b/6b),  $\delta$ 133.43 (C-1b),  $\delta$ 133.98 (C-1a),  $\delta$ 141.67 (C-9a),  $\delta$ 142.66 (C-9b),  $\delta$ 155.62 (C-4b),  $\delta$ 157 (C-11a),  $\delta$ 158.02 (C-13a),  $\delta$ 159.21 (C-11b),  $\delta$ 159.97 (C-4a),  $\delta$ 160.13 (C-13b); ESMS (negative ion mode) m/z [M-H]<sup>-</sup> 469(50); ES MS (positive ion mode) m/z 360 (20). These data are in agreement with those found in the literature (Tanaka et al. 2000).

(3) UV (MeOH,  $\lambda_{\max}$ ) 199, 283nm; <sup>1</sup>H-NMR (500MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 4.05 (1H, d, J=5.3, H-7b),  $\delta$ 4.29 (1H, d, J=4.4, H-8a),  $\delta$ 4.72 (1H, d, J=5.3, H-8b),  $\delta$ 5.45 (1H, d, J=4.4, H-7a),  $\delta$ 6.05 (1H, d, J=2.0, H-14b),  $\delta$ 6.20 (3H, d, J=2.0, H-10a/12a/14a),  $\delta$ 6.25 (1H, d, J=2.0, H-12b),  $\delta$ 6.61 (2H, d, J=8.3, H-3b/5b),  $\delta$ 6.73 (2H, d, J=8.3, H-3a/5a),  $\delta$ 6.88 (2H, d, J=8.3, H-2b/6b),  $\delta$ 7.10 (2H, d, J=8.3, H-2a/6a); <sup>13</sup>C-NMR (125MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 56.47 (C-8a),  $\delta$ 74.0 (C-8b),  $\delta$ 75.16 (C-7b),  $\delta$ 93.39 (C-7a),  $\delta$ 95.83 (C-12b),  $\delta$ 100.96 (C-12a),  $\delta$ 106.2 (C-10a/14a),  $\delta$ 106.75 (C-14b),  $\delta$ 113.77 (C-3b/5b),  $\delta$ 114.86 (C-3b/5b),  $\delta$ 119.28 (C-10b),  $\delta$ 126.5 (C-2a/6a),  $\delta$ 128.79 (C-2b/6b),  $\delta$ 131.70 (C-1b),  $\delta$ 133.21 (C-1a),  $\delta$ 139.5 (C-9b),  $\delta$ 147.21 (C-9a),  $\delta$ 157.21 (C-4a),  $\delta$ 156.27 (C-4b),  $\delta$ 157.89 (C-13b),  $\delta$ 158.59 (C-11a),  $\delta$ 158.89 (C-13a),  $\delta$ 160.65 (C-11b); ESMS (negative ion mode) m/z 487[M-H]<sup>-</sup> (100), ESMS (positive ion mode) m/z 453 (50), 360 (25). These data are in agreement with those found in the literature (Oshima et al. 1995).

(4) UV (MeOH,  $\lambda_{\max}$ ) 232, 282nm; <sup>1</sup>H-NMR (500MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 4.03 (1H, d, J=11.7, H-8a),  $\delta$ 5.36 (1H, d, J=4.9, H-7b),  $\delta$ 5.39 (1H, d, J=4.9, H-8b),  $\delta$ 5.70 (1H, d, J=11.7, H-7a),  $\delta$ 6.11 (1H, d, J=1.9, H-10a),  $\delta$ 6.13 (1H, d, J=2.4, H-12b),  $\delta$ 6.32 (1H, d, J=1.9, H-12a),  $\delta$ 6.53 (1H, d, J=2.4, H-10b),  $\delta$ 6.58 (2H, d, J=8.8, H-3b/5b),  $\delta$ 6.69 (2H, d, J=8.8, H-3a/5a),  $\delta$ 6.83 (2H, d, J=8.8, H-2b/6b),  $\delta$ 7.03 (2H, d, J=8.8, H-2a/6a); <sup>13</sup>C-NMR (125MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 42.88 (C-7b),  $\delta$ 48.56 (C-8a),  $\delta$ 70.35 (C-8b),  $\delta$ 87.96 (C-7a),  $\delta$ 96.30 (C-12b),  $\delta$ 100.33 (C-12a),  $\delta$ 104.43 (C-10a),  $\delta$ 109.61 (C-10b),  $\delta$ 114.37 (C-3b/5b),  $\delta$ 114.95 (C-3a/5a),  $\delta$ 117.93 (C-14a),  $\delta$ 118.56 (C-14b),  $\delta$ 127.75 (C-2b/6b),  $\delta$ 128.93 (C-2a/6a),  $\delta$ 129.69 (C-1a),  $\delta$ 131.85 (C-1b),  $\delta$ 138.72 (C-9b),  $\delta$ 142.06 (C-9a),  $\delta$ 154.88 (C-4b),  $\delta$ 155.94 (C-13a),  $\delta$ 157.66 (C-4a),  $\delta$ 158 (C-11a),  $\delta$ 159.12 (C-11b),  $\delta$ 159.47 (C-13b); ESMS (negative ion mode) m/z 469[M-H]<sup>-</sup> (70); ESMS (positive ion mode) m/z 360 (60). These data are in agreement with those found in the literature (Seo et al. 1999).

(5) UV (MeOH)  $\lambda_{\max}$  200 nm, 280 nm; <sup>1</sup>H-NMR (500MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 3.92 (1H, d, J=12.7, H-8b),  $\delta$ 4.22 (1H, d, J=12.2, H-8a),  $\delta$ 5.18 (1H, d, J=2.4, H-14b),

$\delta$ 5.72 (1H, d, J=2.4, H-12b),  $\delta$ 5.75 (1H, d, J=12.2, H-7a),  $\delta$ 5.80 (1H, d, J=12.7, H-7b),  $\delta$ 6.28 (1H, d, J=1.9, H-14a),  $\delta$ 6.54 (1H, d, J=1.9, H-12a),  $\delta$ 6.56 (2H, d, J=9.3, H-3b/5b),  $\delta$ 6.79 (2H, d, J=8.8, H-3a/5a),  $\delta$ 6.91 (2H, d, J=9.3, H-2b/6b),  $\delta$ 7.15 (2H, d, J=8.8, H-2a/6a); <sup>13</sup>C-NMR (125MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 41.28 (C-7b),  $\delta$ 48.31 (C-8b),  $\delta$ 49.84 (C-8a),  $\delta$ 88.29 (C-7a),  $\delta$ 95.33 (C-12b),  $\delta$ 100.42 (C-14a),  $\delta$ 101.23 (C-12a),  $\delta$ 111.33 (C-14b),  $\delta$ 115.29 (C-3b/5b),  $\delta$ 116.09 (C-2a/6a),  $\delta$ 118.07 (C-10b),  $\delta$ 121.25 (C-10a),  $\delta$ 129.38 (C-2b/6b),  $\delta$ 130.37 (C-2a),  $\delta$ 131.07 (C-1a),  $\delta$ 135.31 (C-1b),  $\delta$ 140.56 (C-9b),  $\delta$ 142.50 (C-9a),  $\delta$ 155.71 (C-4b),  $\delta$ 157.25 (C-13a),  $\delta$ 157.34 (C-13b),  $\delta$ 158.56 (C-4a),  $\delta$ 158.91 (C-11a),  $\delta$ 159.33 (C-11b); ESMS (positive ion mode) m/z 907 [M+H]<sup>+</sup> (100), 453 (73); ESMS (negative ion mode) m/z 905 [M-H]<sup>-</sup> (100). These data are in agreement with those found in the literature (Kawabata et al. 1992).

(6) UV (MeOH,  $\lambda_{\max}$ ) 237, 284nm; <sup>1</sup>H-NMR (500MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 3.14 (1H, brd, J=12.2, H-8b),  $\delta$ 4.04 (1H, t, J=11.2, H-7c),  $\delta$ 4.30 (1H, d, J=12.2, H-8a),  $\delta$ 4.39 (1H, d, J=12.2, H-8c),  $\delta$ 4.60 (1H, d, J=5.9, H-8d),  $\delta$ 5.07 (1H, d, J=2.9, H-7b),  $\delta$ 5.27 (1H, d, J=5.9, H-7d),  $\delta$ 5.70 (1H, d, J=12.7, H-7a),  $\delta$ 5.94 (1H, d, J=2.4, H-14a),  $\delta$ 6.03 (1H, s, H-12b),  $\delta$ 6.04 (2H, brs, H-10d/14d),  $\delta$ 6.16 (1H, d, J=2.4 H-12a),  $\delta$ 6.15 (1H, d, J=2.0, H-12c),  $\delta$ 6.18 (1H, t, J=2.3, H-12d),  $\delta$ 6.42 (1H, d, J=2.0, H-14c),  $\delta$ 6.43 (2H, d, J=8.3, H-2c/6c),  $\delta$ 6.47 (2H, d, J=8.3, H-3c/5c),  $\delta$ 6.67 (2H, d, J=8.7, H-3b/5b),  $\delta$ 6.72 (2H, d, J=8.8, H-3a/5a),  $\delta$ 6.76 (2H, d, J=8.8, H-3d/5d),  $\delta$ 7.11 (2H, d, J=8.7, H-2b/6b),  $\delta$ 7.14 (2H, d, J=8.8, H-2a/6a),  $\delta$ 7.15 (2H, d, J=8.8, H-2d/6d); <sup>13</sup>C-NMR (125MHz, CD<sub>3</sub>OD, ppm)  $\delta$ 36.5 (C-7b),  $\delta$ 47.4 (C-8a),  $\delta$ 49.4 (C-8c),  $\delta$ 52.1 (C-8b),  $\delta$ 56.4 (C-8d),  $\delta$ 57.4 (C-7c),  $\delta$ 89.9 (C-7a),  $\delta$ 95.6 (C-12c),  $\delta$ 94.7 (C-7d),  $\delta$ 95.3 (C-12b),  $\delta$ 100.6 (C-12d),  $\delta$ 100.9 (C-12a),  $\delta$ 104.2 (C-14a),  $\delta$ 105.9 (C-14c),  $\delta$ 106.4 (C-10d/14d),  $\delta$ 114.2 (C-3b/5b),  $\delta$ 114.2 (C-3d/5d),  $\delta$ 114.8 (C-10b),  $\delta$ 114.8 (C-3c/5c),  $\delta$ 115.2 (C-3a/5a),  $\delta$ 121.9 (C-14b),  $\delta$ 122.1 (C-10c),  $\delta$ 124.2 (C-10a),  $\delta$ 127.5 (C-2d/6d),  $\delta$ 128.2 (C-2c/6c),  $\delta$ 129.8 (C-2b/6b),  $\delta$ 130.1 (C-2a),  $\delta$ 131.2 (C-1c),  $\delta$ 130.72 (C-1a),  $\delta$ 132.6 (C-1b),  $\delta$ 133.3 (C-1d),  $\delta$ 140.8 (C-9a),  $\delta$ 141.5 (C-9c),  $\delta$ 143.0 (C-9b),  $\delta$ 147.6 (C-9d),  $\delta$ 154.4 (C-13b),  $\delta$ 154.4 (C-4b),  $\delta$ 154.67 (C-11a),  $\delta$ 154.9 (C-4c),  $\delta$ 155.4 (C-13a),  $\delta$ 157.5 (C-11b),  $\delta$ 157.5 (C-4d),  $\delta$ 158.0 (C-4a),  $\delta$ 158.9 (C-13c),  $\delta$ 159.7 (C-11d/13d),  $\delta$ 160.9 (C-11c); ESMS (positive ion mode) m/z 907 [M+H]<sup>+</sup> (100), 813 (20), 571 (10); ESMS (negative ion mode) m/z 905 [M-H]<sup>-</sup> (100). These data are in agreement with those found in the literature (Tanaka et al. 2000).

(7) UV (MeOH,  $\lambda_{\max}$ ) 202 nm, 290 nm;  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 3.62 (1H, m, H-5''),  $\delta$ 3.79 (1H, d, J=2.0, H-3''),  $\delta$ 3.89 (1H, d, J=1.0, H-2''),  $\delta$ 4.19 (1H, s, H-1''),  $\delta$ 4.25 (1H, m, H-4''),  $\delta$ 4.87 (1H, d, J=12.2, H-3),  $\delta$ 5.01 (1H, d, J=12.2, H-2),  $\delta$ 5.90 (1H, d, J=2.0, H-8),  $\delta$ 5.93 (1H, d, J=2.0, H-6),  $\delta$ 6.83 (1H, d, J=2.0, H-5'),  $\delta$ 6.89 (1H, d, J=2.0, H-6'),  $\delta$ 7.01 (1H, d, J=2.0, H-2');  $^{13}\text{C-NMR}$  (125MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 61.68 (C-5''),  $\delta$ 73.67 (C-3),  $\delta$ 77.97 (C-3''),  $\delta$ 79.85 (C-2''),  $\delta$ 82.63 (C-2),  $\delta$ 97.73 (C-4''),  $\delta$ 95.32 (C-8),  $\delta$ 96.24 (C-6),  $\delta$ 101.01 (C-10),  $\delta$ 106.64 (C-1''),  $\delta$ 114.62 (C-2'),  $\delta$ 115.06 (C-5'),  $\delta$ 119.51 (C-6'),  $\delta$ 127.94 (C-1'),  $\delta$ 144.84 (C-3'),  $\delta$ 146.64 (C-4'),  $\delta$ 163.25 (C-9),  $\delta$ 164.17 (C-5),  $\delta$ 167.96 (C-7),  $\delta$ 196.38 (C-4); ES-MS (negative ion mode) m/z 435  $[\text{M-H}]^-$  (100), 303 (30). These data are in agreement with those found in the literature (Ishimaru et al. 1995).

(8) UV (MeOH,  $\lambda_{\max}$ ) 202 nm, 290 nm;  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 3.08 (1H, dd, J=3.4, 8.3, H-5''),  $\delta$ 3.23 (1H, m, H-3''),  $\delta$ 3.24 (1H, m, H-2''),  $\delta$ 3.49 (1H, m, H-4''),  $\delta$ 3.88 (1H, d, J=6.8, H-1''),  $\delta$ 3.94 (1H, dd, J=4.4, 5.9, H-5''),  $\delta$ 4.75 (1H, d, J=10.3, H-3),  $\delta$ 5.20 (1H, d, J=10.3, H-2),  $\delta$ 5.90 (1H, d, J=2.4, H-6),  $\delta$ 5.92 (1H, d, J=2.4, H-8),  $\delta$ 6.80 (1H, d, J=2.0, H-5'),  $\delta$ 6.83 (1H, d, J=2.0, H-6'),  $\delta$ 6.94 (1H, d, J=2.0, H-2');  $^{13}\text{C-NMR}$  (125MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 64.70 (C-5''),  $\delta$ 69.65 (C-4''),  $\delta$ 72.15 (C-2''),  $\delta$ 74.43 (C-3''),  $\delta$ 76.30 (C-3),  $\delta$ 82.31 (C-2),  $\delta$ 95.12 (C-8),  $\delta$ 97.14 (C-6),  $\delta$ 100.87 (C-10),  $\delta$ 101.79 (C-1''),  $\delta$ 114.42 (C-2'),  $\delta$ 115.07 (C-5'),  $\delta$ 119.49 (C-6'),  $\delta$ 127.56 (C-1'),  $\delta$ 146.11 (C-4'),  $\delta$ 146.34 (C-3'),  $\delta$ 162.25 (C-9),  $\delta$ 164.0 (C-5),  $\delta$ 167.87 (C-7),  $\delta$ 194.99 (C-4); ES-MS (negative ion mode) m/z 435  $[\text{M-H}]^-$  (100), 303 (10). These data are in agreement with those found in the literature (Chosson et al. 1998).

(9) UV (MeOH,  $\lambda_{\max}$ ) 204 nm, 255 nm, 347 nm;  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 0.94 (1H, d, J=6.35, H-6''),  $\delta$ 3.34 (1H, dd, J=3.90, H-4''),  $\delta$ 3.44 (1H, dd, J=6.5, H-5''),  $\delta$ 3.78 (1H, dd, J=3.4, H-3''),  $\delta$ 4.22 (1H, dd, J=2.0, H-2''),  $\delta$ 5.37 (1H, d, J=2.0, H-1''),  $\delta$ 6.18 (1H, d, J=2.4, H-6),  $\delta$ 6.38 (1H, d, J=2.4, H-8),  $\delta$ 6.90 (H-5'),  $\delta$ 7.30 (H-6'),  $\delta$ 7.34 (H-2');  $^{13}\text{C-NMR}$  (125MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 16.41 (C-6''),  $\delta$ 70.64 (C-2''),  $\delta$ 70.80 (C-5''),  $\delta$ 70.83 (C-3''),  $\delta$ 72.04 (C-4''),  $\delta$ 93.55 (C-8),  $\delta$ 98.59 (C-6),  $\delta$ 102.28 (C-1''),  $\delta$ 104.59 (C-10),  $\delta$ 115.18 (C-5'),  $\delta$ 115.75 (C-6'),  $\delta$ 121.59 (C-1'),  $\delta$ 121.63 (C-2'),  $\delta$ 134.52 (C-3),  $\delta$ 145.21 (C-3'),  $\delta$ 148.32 (C-4'),  $\delta$ 157.2 (C-5),  $\delta$ 158.18 (C-2),  $\delta$ 161.86 (C-9),  $\delta$ 164.52 (C-7),  $\delta$ 180 (C-4); ES-MS (negative ion mode) m/z 447  $[\text{M-H}]^-$  (100), 301 (30); ES-MS (positive ion mode) m/z 303  $[\text{M+H}]^+$  (100). These data are in agreement with those found in the literature (Zhang et al. 2003).

(10) UV (MeOH,  $\lambda_{\max}$ ) 207 nm, 261 nm, 349 nm;  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 0.94 (1H, d, J=6.34, H-6''),  $\delta$ 3.34 (1H, m, H-4''),  $\delta$ 3.53 (1H, dd, J=6.35, H-5''),  $\delta$ 3.78 (1H, dd, J=3.40, H-3''),  $\delta$ 4.21 (1H, dd, J=2.0, H-2''),  $\delta$ 5.31 (1H, br d, H-1''),  $\delta$ 6.21 (1H, d, J=2.0, H-6),  $\delta$ 6.36 (1H, d, J=2.0, H-8),  $\delta$ 6.95 (1H, s, H-2'/6');  $^{13}\text{C-NMR}$  (125MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 16.45 (C-6''),  $\delta$ 70.59 (C-2''),  $\delta$ 70.77 (C-5''),  $\delta$ 70.84 (C-3''),  $\delta$ 72.05 (C-4''),  $\delta$ 93.47 (C-8),  $\delta$ 98.54 (C-6),  $\delta$ 102.4 (C-1''),  $\delta$ 104.90 (C-10),  $\delta$ 108.24 (C-2'/C-6'),  $\delta$ 122.05 (C-1'),  $\delta$ 136.6 (C-3),  $\delta$ 138.04 (C-4'),  $\delta$ 145.82 (C-3'/C-5'),  $\delta$ 157.63 (C-9),  $\delta$ 158.03 (C-2),  $\delta$ 162.09 (C-5),  $\delta$ 164.71 (C-7),  $\delta$ 179.75 (C-4); ES-MS (negative ion mode) m/z 463  $[\text{M-H}]^-$  (100), 317  $[\text{M+1}]^+$  (12). These data are in agreement with those found in the literature (Addae-Mensah & Achenbach 1985).

(11) UV (MeOH,  $\lambda_{\max}$ ) 212 nm, 267 nm, 290 nm, 347 nm;  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 1.19 (1H, d, J=6.1, H-6''),  $\delta$ 3.30 (H-4''),  $\delta$ 3.55 (1H, dd, H-2''),  $\delta$ 3.66 (1H, dd, H-3''),  $\delta$ 4.05 (1H, br s, H-1''),  $\delta$ 4.25 (1H, m, H-5''),  $\delta$ 4.52 (1H, d, J=2.0, H-3),  $\delta$ 5.07 (1H, d, J=11.0, H-2),  $\delta$ 5.90 (1H, d, J=2.2, H-8),  $\delta$ 5.91 (1H, d, J=2.2, H-6),  $\delta$ 6.95 (1H, d, J=2.2, H-8),  $\delta$ 6.81 (1H, d, J=8.3, H-5'),  $\delta$ 6.84 (1H, dd, J=2.3, 8.3, H-6'),  $\delta$ 6.95 (1H, d, J=2.0, H-2');  $^{13}\text{C-NMR}$  (125MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 16.6 (C-6''),  $\delta$ 69.29 (C-5''),  $\delta$ 70.44 (C-2''),  $\delta$ 70.92 (C-3''),  $\delta$ 72.51 (C-4''),  $\delta$ 77.30 (C-3),  $\delta$ 82.65 (C-2),  $\delta$ 95.25 (C-8),  $\delta$ 96.21 (C-6),  $\delta$ 100.86 (C-1''),  $\delta$ 101.27 (C-10),  $\delta$ 114.21 (C-2'),  $\delta$ 115.10 (C-5'),  $\delta$ 119.18 (C-6'),  $\delta$ 127.87 (C-1'),  $\delta$ 144.99 (C-3'),  $\delta$ 146.26 (C-4'),  $\delta$ 163.02 (C-9),  $\delta$ 164.22 (C-5),  $\delta$ 167.81 (C-7),  $\delta$ 194.59 (C-4); ES-MS (negative ion mode) m/z 449  $[\text{M-H}]^-$  (100); ESMS (positive ion mode) m/z 303  $[\text{M+H}]^+$  (100). The data are in agreement with those found in the literature (Agrawal 1989).

(12) UV (MeOH,  $\lambda_{\max}$ ) 212 nm, 267 nm, 290 nm, 347 nm;  $^1\text{H-NMR}$  (500MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 3.10 (1H, dd, J=9.4, 2.5, H-6''),  $\delta$ 3.40 (H-3''),  $\delta$ 3.51 (1H, m, H-4''),  $\delta$ 3.52 (1H, m, H-2''),  $\delta$ 3.78 (1H, dd, J=5.5, H-5''),  $\delta$ 5.19 (1H, d, J=8.0, H-1''),  $\delta$ 6.21 (1H, d, J=2.2, H-8),  $\delta$ 6.41 (1H, d, J=2.2, H-6),  $\delta$ 6.86 (1H, d, J=8.3, H-5'),  $\delta$ 7.59 (1H, dd, J=2.2, 8.3, H-6'),  $\delta$ 7.61 (1H, d, J=2.2, H-2');  $^{13}\text{C-NMR}$  (125MHz,  $\text{CD}_3\text{OD}$ , ppm)  $\delta$ 65.99 (C-6''),  $\delta$ 65.99 (C-5''),  $\delta$ 69.81 (C-4''),  $\delta$ 73.98 (C-2''),  $\delta$ 76.40 (C-3''),  $\delta$ 93.52 (C-6),  $\delta$ 98.73 (C-8),  $\delta$ 103.33 (C-1''),  $\delta$ 104.4 (C-10),  $\delta$ 114.86 (C-5'),  $\delta$ 116.10 (C-2'),  $\delta$ 121.96 (C-6'),  $\delta$ 122.86 (C-1'),  $\delta$ 134.07 (C-3),  $\delta$ 146.9 (C-3'),  $\delta$ 148.61 (C-4'),  $\delta$ 157.2 (C-5),  $\delta$ 157.57 (C-2),  $\delta$ 161.7 (C-9),  $\delta$ 165.07 (C-7),  $\delta$ 180 (C-4); ES-MS (negative ion mode) m/z 433  $[\text{M-H}]^-$  (80), 301 (10). These data are in agreement with those found in the literature (Agrawal 1989).

## RESULTS AND DISCUSSION

The ethyl acetate extract of the leaves of *P. campechiana* was subjected to Silica and Sephadex LH-20 column chromatography and HPLC resulting to the isolation of 12 compounds. Their structures were established from spectral analysis including 1D and 2D NMR experiments.

The stilbenoids isolated were the dimers ampelopsin B (1) (Seo et al. 1999), balanocarpol (2) (Tanaka et al. 2000b),  $\epsilon$ -viniferin diol (3) (Oshima et al. 1995b), ampelopsin A (4) (Seo et al. 1999) as shown in Figure 1 along with 2 tetramers hopeaphenol (5) (Kawabata et al. 1992) and vaticaphenol A (6) (Tanaka et al. 2000b) shown in Figure 2. Aside from these stilbenes, six flavonoid

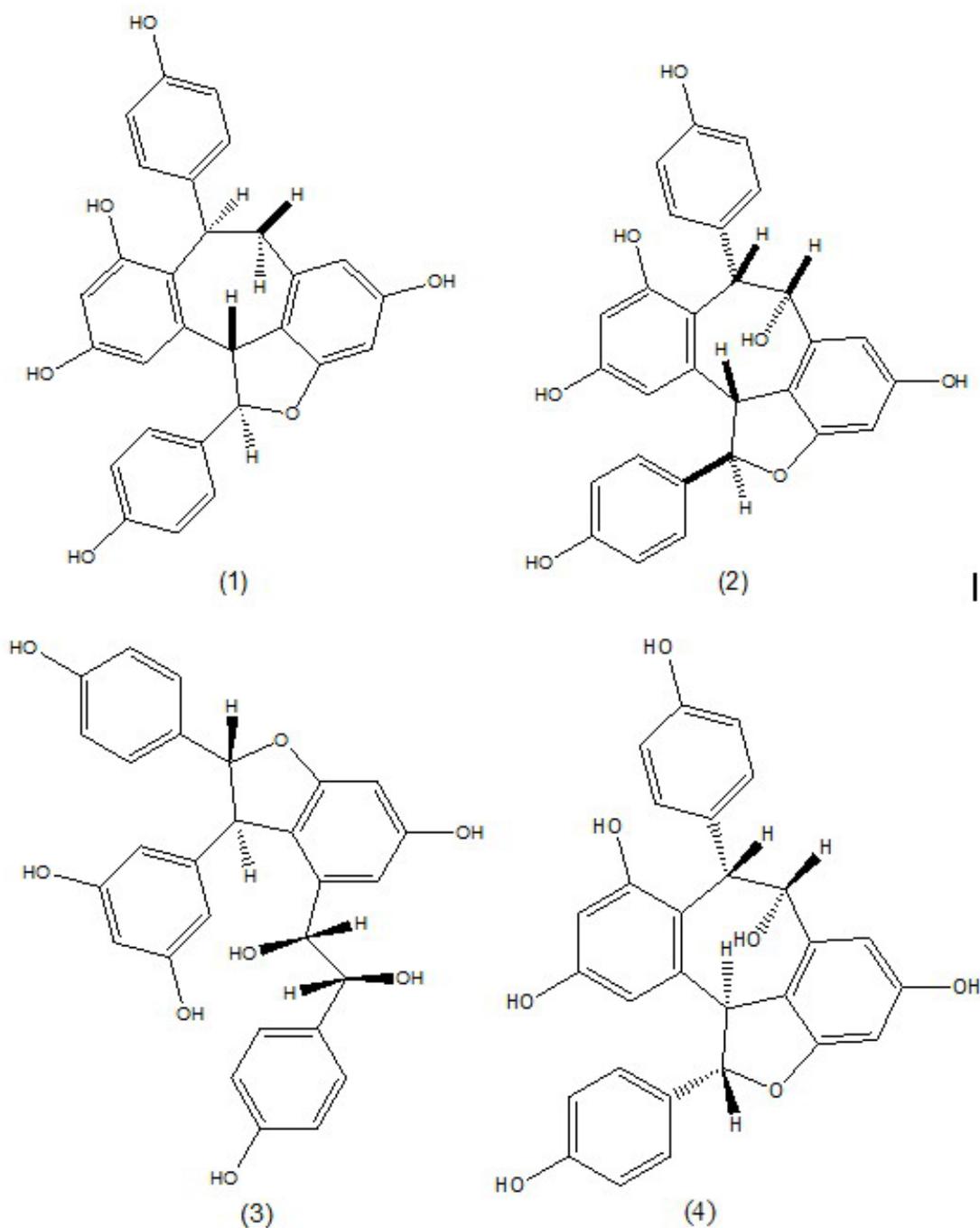


Figure 1. The four stilbene dimers (1 – 4) isolated from the ethyl acetate extract of *P. campechiana*.

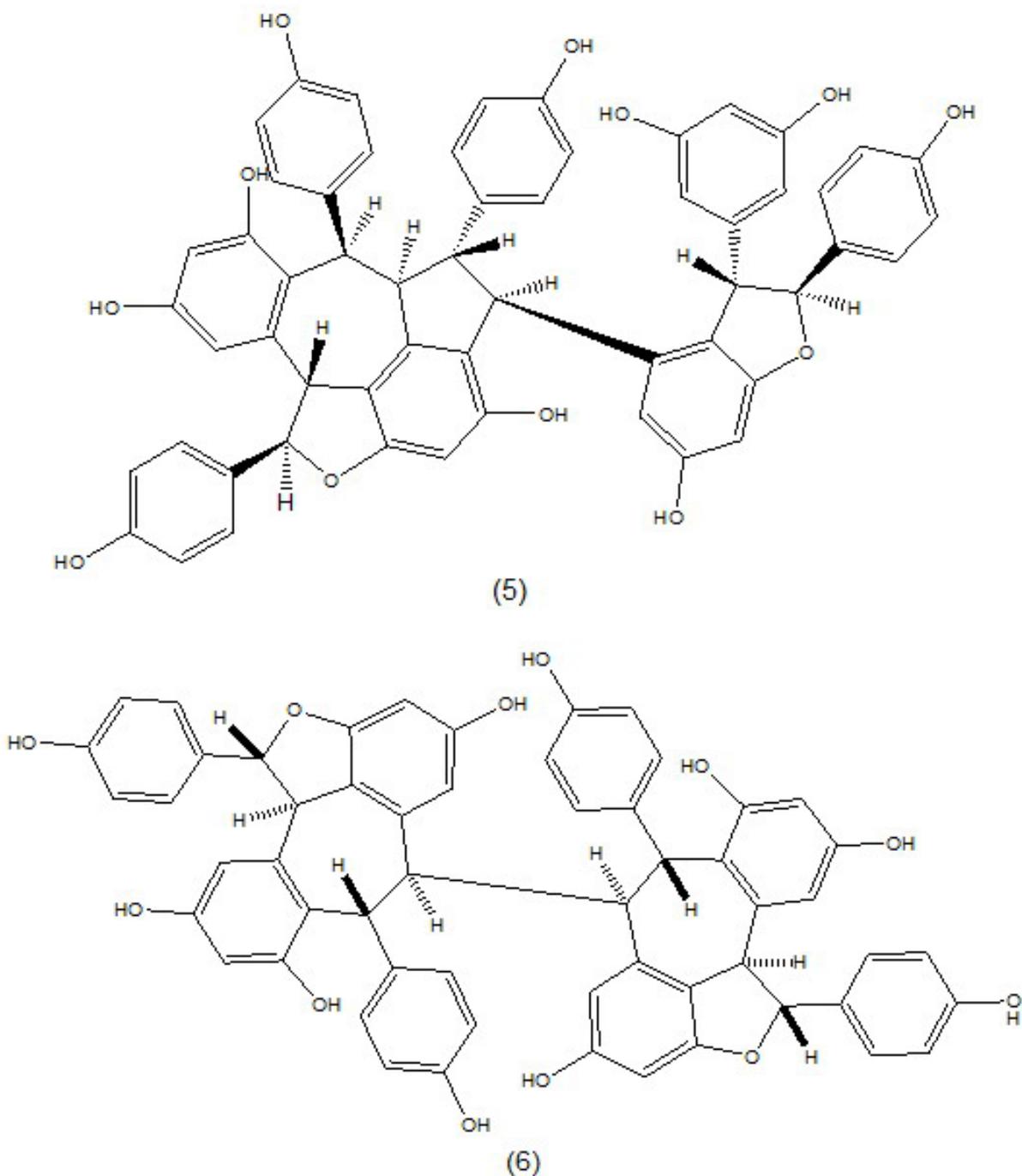


Figure 2. The two stilbene tetramers (5 – 6) isolated from the ethyl acetate extract of *P. campechiana*.

glycosides were also purified and identified as taxifolin 3-O- $\alpha$ -arabinofuranoside (7) (Ishimaru et al. 1995), trans-taxifolin 3-O- $\alpha$ -arabinopyranoside (8) (Chosson et al. 1998), quercetin 3-O- $\alpha$ -rhamnopyranoside (9) (Zhang et al. 2003), myricetin 3-O- $\alpha$ -rhamnopyranoside or myricitrin (10) (Addae-Mensah & Achenbach 1985)

and a mixture of taxifolin 3-O- $\alpha$ -rhamnopyranoside (11) (Agrawal 1989) and quercetin 3-O- $\beta$ -arabinopyranoside (12) (Agrawal 1989) as seen in Figure 3.

All the compounds isolated were evaluated for their antimitotic activity in the Institute of Chemistry

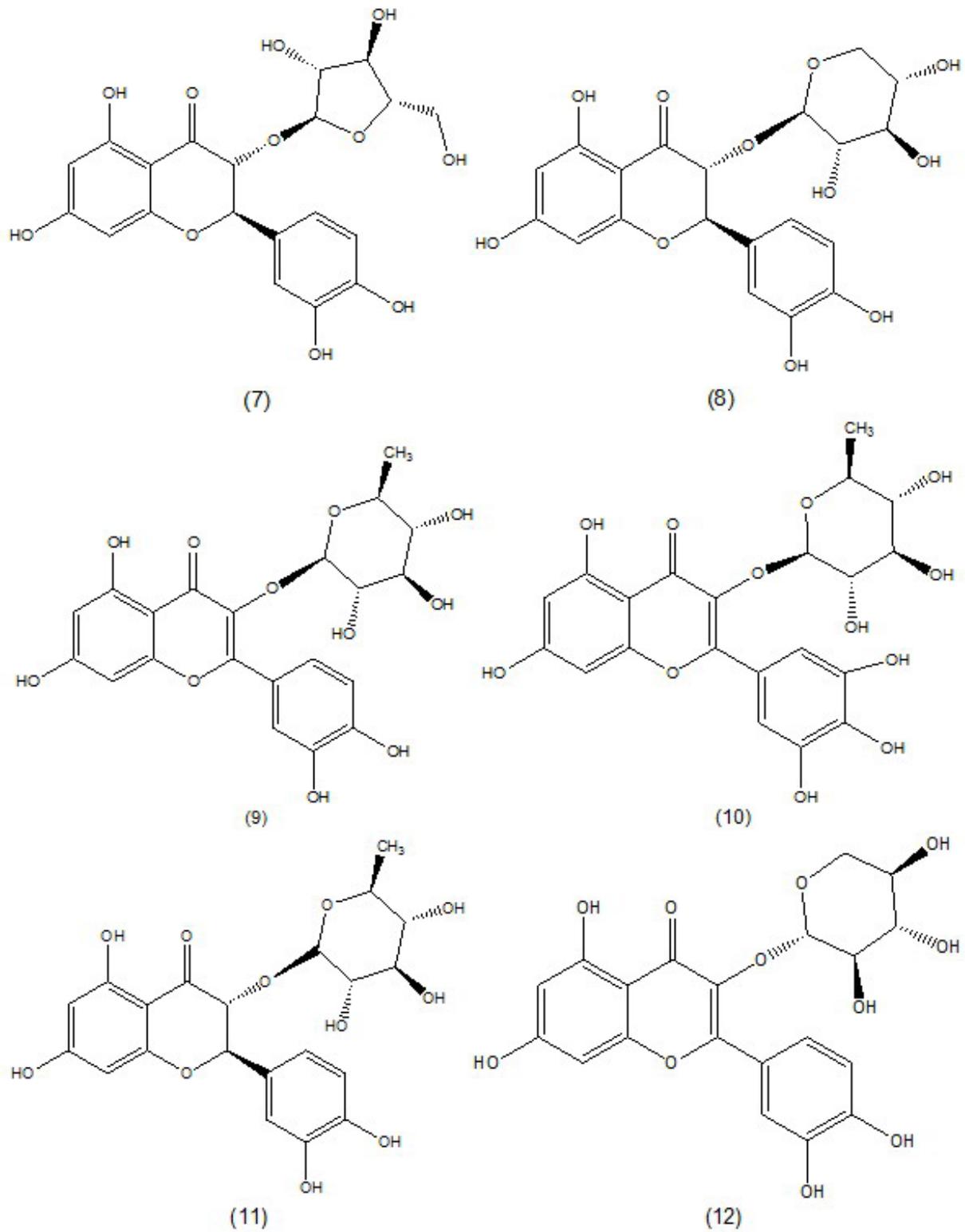
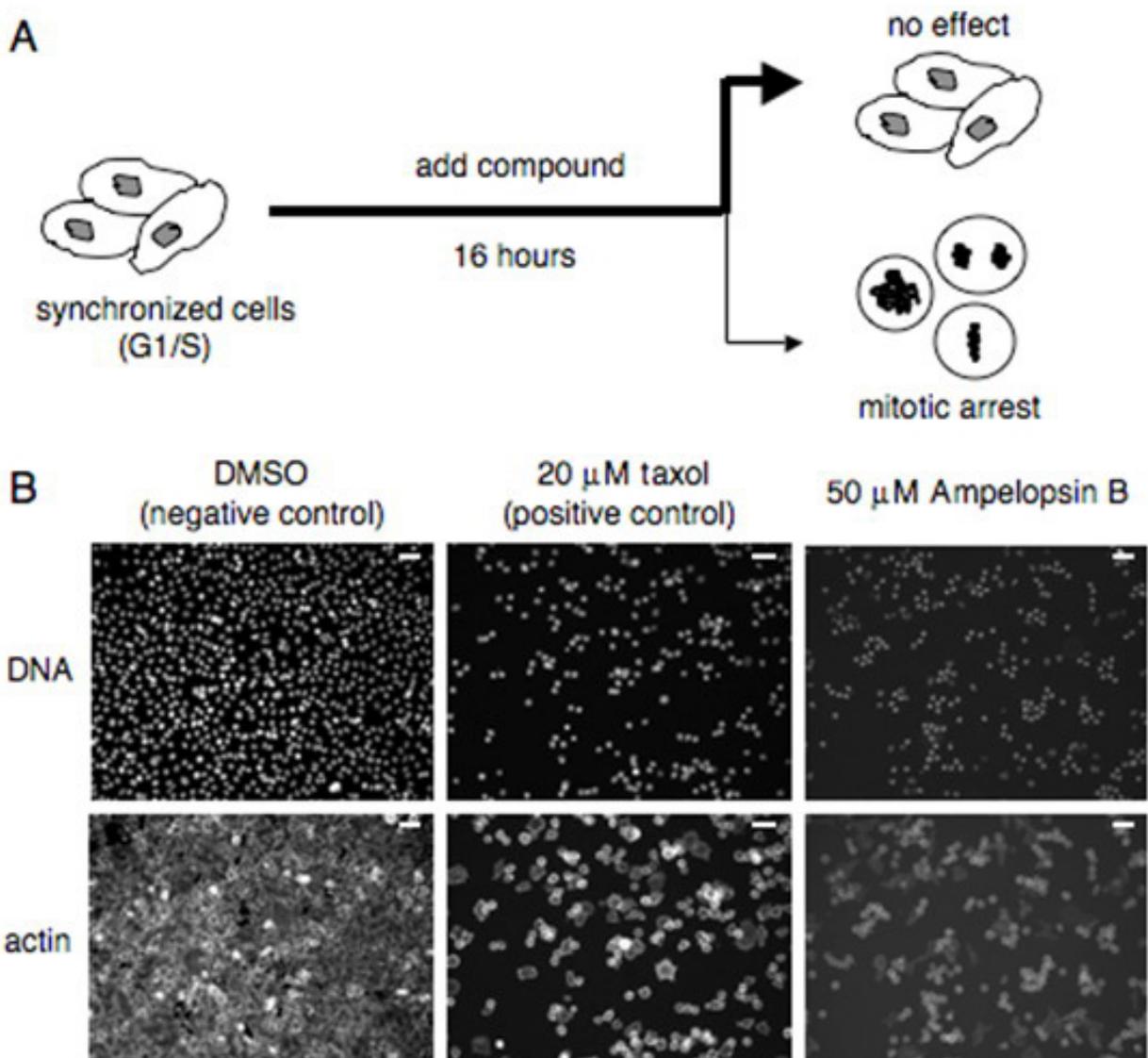


Figure 3. The six flavonoid glycosides (7 – 12) isolated from *P. campechiana*

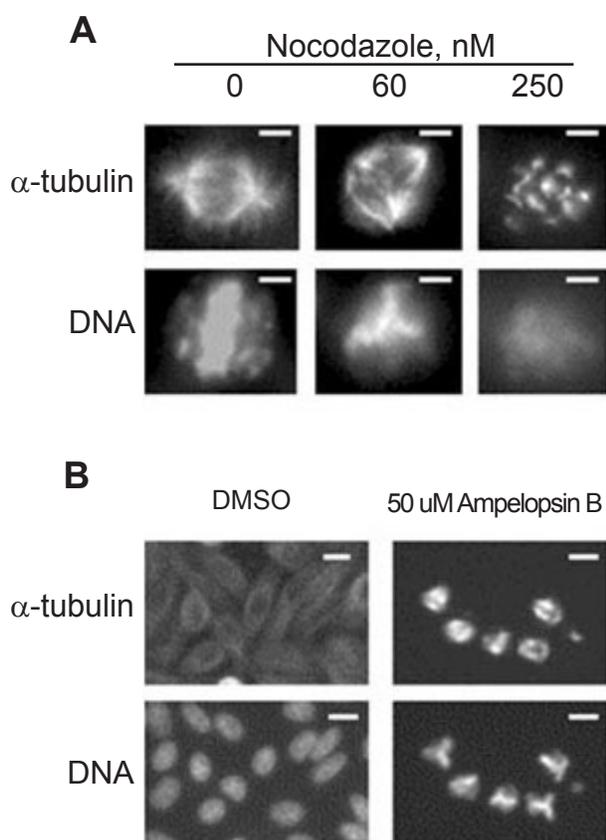


**Figure 4.** A high-content screen to identify anti-mitotic activity. A) Cartoon depicting screening strategy. Cells synchronized in G1/S were incubated with compounds for 16 h, prior to fixation and staining for actin and DNA, followed by automated image acquisition and analysis. B) Representative screening images depicting the effects of negative (DMSO) and positive (20  $\mu$ M Taxol) controls, and 50  $\mu$ M Ampelopsin B on actin and DNA within HeLa cells. Scale bar = 100  $\mu$ m.

and Cell Biology, Harvard Medical School Screening Facility. A phenotypic screen was employed in which synchronized HeLa cells were plated in 384 well-plates and the compounds were added to a final concentration of 50  $\mu$ M. After 16 h cells were fixed and stained to visualize DNA and actin and images were acquired using automated microscopy. Images were then analyzed by eye to identify compounds that resulted in mitotic arrest (Yarrow et al. 2003, Mitchison 2004) (Figure 4A). Mitotic inhibitors usually reveal themselves by causing cell rounding, evidenced by analysis of the actin cytoskeleton. From the

12 compounds, only ampelopsin B was scored as positive in the primary screen and demonstrated characteristic cell rounding and condensation of DNA seen with HeLa cells arrested during mitosis (Figure 4B). The actin image showed rounded-up cells, in contrast to the elongated shape observed in the absence of Ampelopsin B. The DNA image showed bright condensed chromosomes similar to that seen in the positive control conditions (treatment with the known anti-mitotic agent taxol), in contrast to the interphase nuclei with diffuse uncondensed chromosomes observed with DMSO treatment (Figure

4B). However, in contrast to treatment with taxol, cells treated with Ampelopsin B showed an altered pattern of DNA condensation. Specifically, the characteristic “bar” structures that represent chromosomes condensed at the metaphase plate were frequently observed (Figure 5), indicating that Ampelopsin B arrested cells during metaphase. Additionally, “Y” shaped structures were also seen when looking at images of DNA from Ampelopsin B-treated cells. These “Y”-shaped structures are also observed when cells are treated with weak microtubule inhibitors (e.g. low doses of nocodazole). To determine whether Ampelopsin B was exerting its anti-mitotic activities through disruption of the microtubule cytoskeleton, additional imaging experiments were



**Figure 5.** Analysis of the microtubule cytoskeleton suggests that Ampelopsin B may function as a microtubule depolymerizer. A) Images of DNA and alpha-tubulin following incubation with nocodazole at a range of concentrations. Note that the image shown at 0 nM nocodazole is a representative image of a cell during metaphase to show a normal bipolar microtubule spindle. Increasing concentrations of nocodazole result in the formation of multipolar spindles (60 nM nocodazole) and then complete disruption of normal spindle architecture (250 nM nocodazole). Scale bar = 5  $\mu$ m. B) Images of DNA and alpha-tubulin following incubation with DMSO (vehicle) or 50  $\mu$ M Ampelopsin B. Note the occurrence of tri-polar microtubule spindles, suggesting that Ampelopsin B is acting as a weak microtubule depolymerizer. Scale bar = 20  $\mu$ m.

performed. Synchronized HeLa cells were plated in 384 well-plates and Ampelopsin B (final concentration: 50  $\mu$ M) and nocodazole (final concentrations: 30 nM, 60 nM, 125 nM and 250 nM) were added. After 16 hours cells were fixed and stained to visualize DNA and microtubules and images were acquired using automated microscopy. Images were then analyzed by eye. In the presence of Ampelopsin B, both bipolar and tripolar microtubule spindles are seen (Figure 5). Tripolar spindles were also frequently observed with low concentrations of nocodazole (Figure 5A), suggesting that Ampelopsin B may be acting as a weak microtubule depolymerizer.

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