Cytotoxic Properties of Oligostilbenoids from the Tree Barks of *Hopea dryobalanoides*

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A new modified stilbene dimer, diptoindonesin D (**1**), was isolated from the acetone extract of the tree bark of *Hopea dryobalanoides*, together with seven known compounds, parviflorol (**2**), (–)-balanocarpol (**3**), heimiol A (**4**), hopeafuran (**5**), (+)-α-viniferin (**6**), vaticanol B (**7**) and (–)-hopeaphenol (**8**). Cytotoxic properties of compounds **1**–**8** were evaluated against murine leukemia P-388 cells. Compound **8** was found to be the most active with IC_{50} of 5.7 μM.

Key words: Diptoindonesin D, *Hopea dryobalanoides*, Murine Leukemia P-388 Cells

Introduction

Dipterocarpaceae plants have been known to be a source of oligostilbenoid compounds (Sotheeswaran and Pasupathy, 1993; Cichewicz and Kouzi, 2002; Hakim, 2002). They include stilbene dimers, trimers, tetramers, hexamers, heptamers and octamers with various molecular frameworks resulting from different oxidative condensation of resveratrol monomer. Some of these compounds show interesting biological activities, such as antibacterial, antiviral, and cytotoxic effects. In previous reports, we have described the isolation and structure elucidation of four new oligostilbenoids, diptoindonesin A, *cis*- and *trans*-diptoindonesin B, and diptoindonesin C, from species of Dipterocarpaceae growing in Indonesia (Aminah *et al*., 2002; Syah *et al*., 2003, 2005). In the course of our research effort on the cytotoxic constituents from the Indonesian plants (Hakim *et al*., 2005; Suhartati *et al.*, 2001; Syah *et al*., 2001; 2004), we now report the isolation of a new modified oligostilbenoid, diptoindonesin D (**1**) (Fig. 1), together with seven known oligostilbenoids, and cytotoxic properties of the constituents isolated from the tree barks of *Hopea dryobalanoides* Miq., a species endemic to Indonesia.

Results and Discussion

The dried powdered tree bark of *H. dryobalanoides* was macerated with acetone, and the acetone extract was fractionated into acetone/diethyl ether soluble and insoluble fractions. The acetone/diethyl ether soluble fraction was fractionated using silica gel chromatography and the fraction containing the least polar of phenolic compounds was purified by the radial chromatographic technique to give compound **1** and parviflorol (**2**) (Tanaka *et al*., 2000a). Using the same methodology, six known oligostilbenoids, (–)-balanocarpol (**3**) (Diyasena *et al*., 1985), heimiol A (**4**) (Weber *et al*., 2001), hopeafuran (**5**) (Tanaka *et al*., 2001), (+)-α-viniferin (**6**) (Pryce and Langcake, 1977), vaticanol B (**7**) (Tanaka *et al*., 2000b), and (–)-hopeaphenol (**8**) (Coggon *et al.*, 1970) (Fig. 1), were also isolated from the more polar fractions.

Compound **1** was isolated as a pale yellow solid and the molecular formula $C_{21}H_{14}O_7$ was assigned to **1** from HRFABMS ($[M+H]^+$ ion at *m/z* 379.0805, calcd. for $C_{21}H_{15}O_7$, 379.0818) suggesting that **1** is a dedihydro derivative of **2** (Tanaka *et al*., 2000a), which is also isolated from this plant. The UV and IR spectra (see Experimental) indicated that **1** contains an extended conjugation of the benzoyl chromophore. The ^{1}H NMR spectrum of **1** (Table I) exhibited a series of aromatic signals

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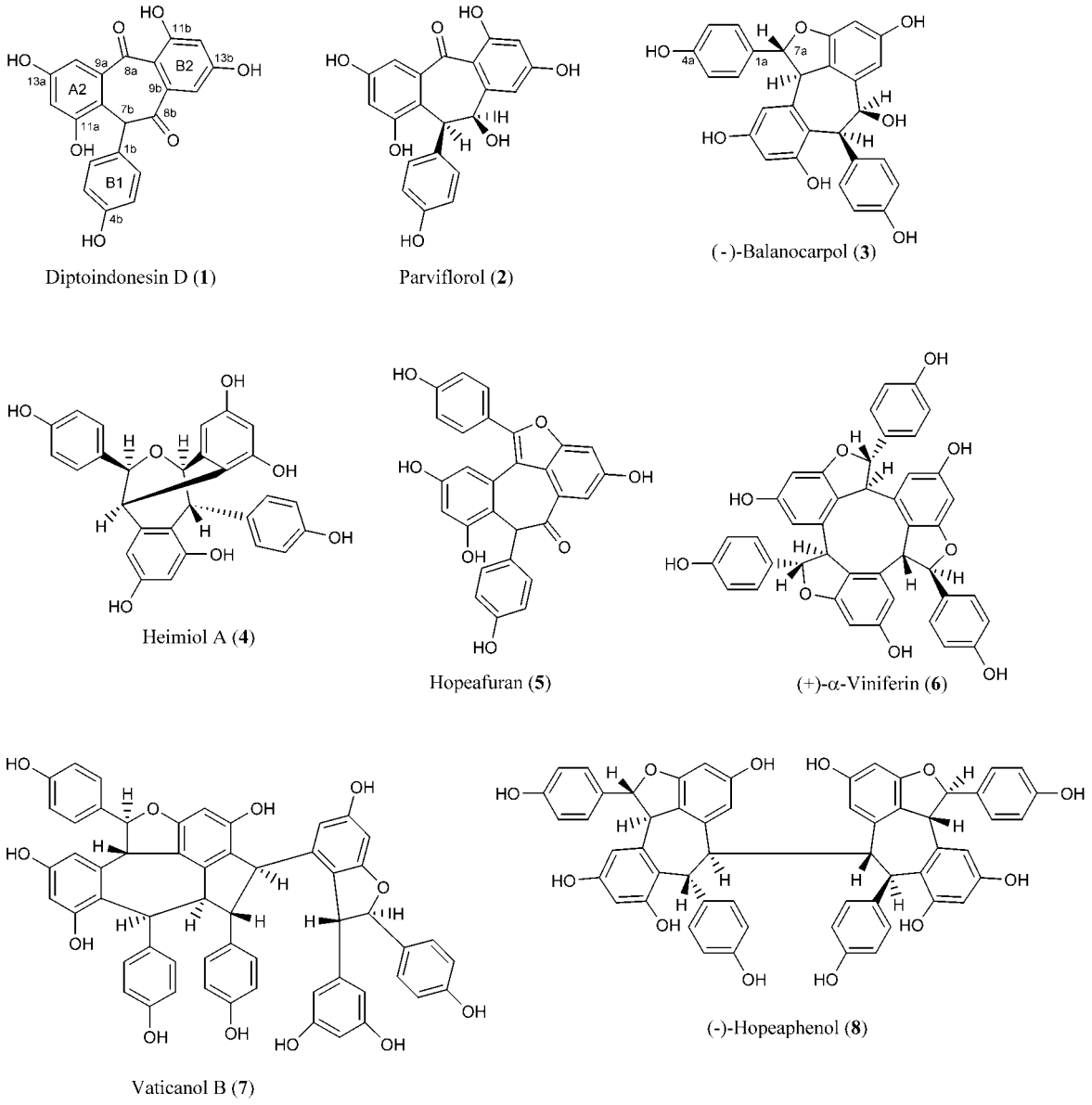

Fig. 1. The constituents isolated from *H. dryobalanoides*.

assignable to one *p*-hydroxyphenyl (δ_H 6.63 and 6.68, ring B1) and two substituted 3,5-dihydroxyphenyl groups (δ_H 6.39, 6.80, 6.90 and 6.92, rings A2 and B2), in addition to a singlet of an aliphatic methine signal at δ_H 5.89 ppm and a chelated phenol group at δ_H 13.68 ppm. The ^{13}C NMR data (Table I), supported by APT and HMQC spectra, especially showed signals for the presence of two conjugated carbonyl carbon atoms (δ_C 196.3 and 197.1) and one aliphatic methine carbon atom (δ_C 54.8), in addition to carbon signals for the three aromatic rings A2, B1 and B2, including those of five oxyaryl carbon atoms. From these data, the basic structure of **1** could be formulated for diptoindonesin D. Support for this structure came from HMBC measurements (Fig. 2), showing, in particular, long range correlations between the proton signals at δ_H 5.89 (H-7b) and 6.92 (H-14b)

Table I. NMR data of compound **1** in acetone-d_6.

No	δ_H (multiplicity, J in Hz)	δ_C
8a	–	197.1
9a	–	142.3
10a	–	110.7
11a	–	156.6
12a	6.80 (d, 2.6)	107.0
13a	–	158.4
14a	6.90 (d, 2.6)	109.8
1b	–	130.0
2b/6b	6.63 (d, 8.8)	128.5
3b/5b	6.68 (d, 8.8)	115.8
4b	–	156.4
7b	5.89 (br s)	54.8
8b	–	196.3
9b	–	139.2
10b	–	111.3
11b	–	166.8
12b	6.39 (d, 2.6)	106.6
13b	–	164.4
14b	6.92 (d, 2.6)	112.5
11b-OH	13.68 (s)	–

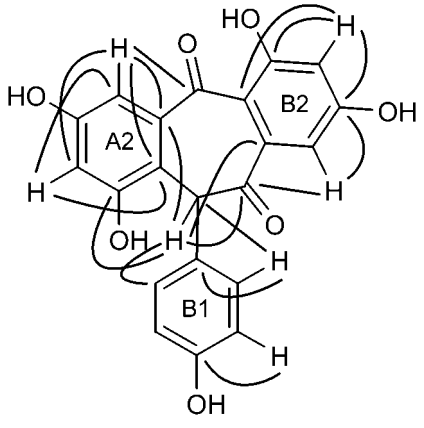

Fig. 2. Selected important HMBC correlations in **1**.

ppm with the carbonyl carbon signal at δ_C 196.3 ppm, confirming the location of one of the carbonyl groups at C-8b. The HMBC spectrum also indicated long range correlations between the proton signal at δ_H 6.90 ppm (H-14a) with the carbonyl signal at δ_C 197.1 ppm, and, together with the presence of the chelated phenol group, supported the location of the second carbonyl group between rings A2 and B2. From these HMBC correlations, therefore, **1** was assigned to be diptoindonesin D. Selected other important HMBC correlations, in support of the structure of **1**, are shown in Fig. 2. The stereochemistry at the methine carbon atom was not determined, however from a biogenetic consideration it would be related to the stereochemistry of the same carbon atom in **2**.

While compounds **3**–**5** are dimer stilbenoids, compounds **1** and **2** could be regarded as modified dimer stilbenoids, and compounds **7** and **8** are tetramersilbenoids. The only trimer stibenoid isolated from this plant is compound **6**. Compounds **6** and **8** are found in many of dipterocarp species, including *Hopea* and *Shorea* plants (Sotheeswaran and Pasupathy, 1993; Hakim, 2002). The oligostilbenoids isolated from *H. dryobalanoides* are, therefore, typical aromatic constituents of *Hopea* species, *i.e.* mainly dimer and tetramer stilbenoids.

The cytotoxic properties of compounds **1**–**8** (Table II) were evaluated against murine leukemia P-388 cells according to the method described previously (Alley *et al*., 1988). Compound **8** was found to be the most active, while compounds **1**, **3** and **6** were moderately cytotoxic, and compounds **2**, **4**, **5** and **7** could be regarded as inactive. Observing cytotoxic properties of compounds **1**–**8** its seemed that there is no definite pattern between the degree of cytotoxicity and the molecular size of oligostilbenoids, consistent with those observed by Ito *et al*. (2003) using several other cell lines. However, the presence of a cycloheptadiene ring, such as in compounds **1**, **3** and **8**, seems to be an important factor for cytotoxicity. Intriguingly, compound **2**, which also contains the same ring, is much less cytotoxic on P-388 cells. Other structural factors, apart from the cycloheptadiene ring, must be required for the cytotoxicity, and less hydroxyl groups could be one of these, as exemplified by the increasing cytotoxicity from compounds **2** and **3** to compound **1**.

Table II. IC_{50} values of compounds **1**–**8** against P-388 cells.

Compound	Oligomer	IC_{50} [μM]
Diptoindonesin D (**1**)	modified dimer	14.5 ± 1.3
Parviflorol (**2**)	modified dimer	145.9 ± 1.8
(–)-Balanocarpol (**3**)	dimer	33.6 ± 8.3
Heimiol A (**4**)	dimer	n.d.*
Hopeafuran (**5**)	dimer	112.6 ± 1.5
(+)-α-Viniferin (**6**)	trimer	25.8 ± 0.7
Vaticanol B (**7**)	tetramer	56.8 ± 2.3
(–)-Hopeaphenol (**8**)	tetramer	5.7 ± 0.3

* n.d., IC_{50} exceeds the concentration range tested that can not be determined.

Experimental

General experimental procedures

UV spectra were measured with a Varian Conc. 100 instrument. IR spectra were determined with a Perkin Elmer FTIR Spectrum One spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL AS400 operating at 400 (^{1}H) and 100 (^{13}C) MHz using residual and deuterated solvent peaks as reference standards. A high resolution mass spectrum was obtained with a VG Autospec mass spectrometer (FAB mode). Vacuum liquid (VLC) and column chromatography were carried out using Merck silica gel 60 GF_{254} and silica gel G60 35–70 mesh. For TLC analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used.

Plant material

The tree barks of *H. dryobalanoides* were collected from the Experimental Garden of Haurbentes, Bogor, West Java, Indonesia, in March 2004, and identified by the staff of Herbarium Bogoriense, Bogor, Indonesia. A voucher specimen was deposited at the Herbarium Bandungense, Institut Teknologi Bandung, Indonesia.

Extraction and isolation

The dried powdered tree bark of *H. dryobalanoides* (1.0 kg) was macerated in acetone. The acetone extract (100 g) was fractionated into acetone/diethyl ether soluble (55 g) and insoluble (45 g) fractions. A portion (25 g) of the acetone/diethyl ether soluble fraction was fractionated by VLC (silica gel, *n*-hexane/EtOAc 7.3 to 2:8, and EtOAc) into six major fractions A–F. Fraction C (3.2 g) was purified either by radial chromatography (silica gel, *n*-hexane/EtOAc 1:1; and *n*-hexane/$CHCl_3$/methanol 4:5:1) to give compounds **1** (85 mg) and **2** (385 mg). Using the same method, fraction D (7.9 g) yielded (–)-balanocarpol (**3**) (240 mg), (–)-hopeaphenol (**8**) (320 mg), and vaticanol B (**7**) (210 mg), while fraction E (6.9 g) gave hopeafuran (**5**) (20 mg), (+)-α-viniferin (**6**) (10 mg), and heimiol A (**4**) (40 mg).

*Diptoindonesin D (**1**)*

Pale yellow solid, m.p. 184–187 °C $[\alpha]_D$ + 318° (MeOH, *c* 0.1). – UV: λ_{max} (MeOH) (log ε) = 203 (4.79), 222 (4.63), 245 (sh, 4.39), 273 (4.28), 375 (4.15) nm; λ_{max} (MeOH+NaOH) = 205 (5.26), 230 (4.68), 296 (4.37), 398 (4.35) nm. – IR (KBr): ν_{max} = 3309, 2956, 1673, 1590 cm^{-1}. – ^{1}H NMR (400 MHz, acetone-d_6): see Table I. – ^{13}C NMR (100 MHz, acetone-d_6): see Table I. – HRFABMS: *m/z*: 379.0805 $[M+H]^+$, calcd. for $C_{21}H_{15}O_7$ 379.0818.

Cytotoxicity assay

The cytotoxicity assay was conducted according to the method described by Alley *et al.* (1988). P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3×10^4 cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH 7.30–7.65). Control wells received only DMSO. The assay was terminated after an 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h of incubation was conducted. Optical density was read by using a microplate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (μM). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

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