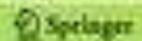
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Chemistry of Natural Compounds

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(-)-AMPELOPSIN F, DIMERSTILBENE COMPOUND FROM *Dryobalanops oblongifolia* AND ANTIMALARIAL ACTIVITY TEST

Indriani,^{1,4} Yoshiaki Takaya,² N. N. T. Puspaningsih,^{3*} and N. S. Aminah^{3*}

The Dipterocarpaceae (dipterocarp) is one of a large family comprising 16 genera, about 600 species of which are widely distributed (76%) in Malaysia and Indonesia. *Dryobalanops* comprises a small number of species. This genus is locally named *kayu kapur* [1, 2]. As other genus in Dipterocarpacea, *Dryobalanops* has been known to be a rich source of phenolic compounds, especially stilbene oligomers [3–6] but there has never been a report on their antimalarial activity.

Previous studies have identified stilbene oligomers from *Dryobalanops oblongifolia* Dyer, namely (–)-ampelopsin A, a compound of dimer stilbenoid, and two compounds of trimer stilbenoid, namely *cis*- and *trans*-diptoindonesin B [3, 4]. This paper reports on a compound of dimer stilbene, (–)-ampelopsin F, from the acetone extract of the tree bark of *D. oblongifolia*. Test of its antimalarial activity showed an IC $_{50}$ value of 0.001 μ g/mL, which makes it promising as an antimalarial compound.

The structure of (–)-ampelopsin F (1) was determined based on physical data and spectroscopic data, including UV, MS, NMR, 2D NMR, and also by comparison with published data. The antimalarial activity test was carried out according to the method of Trager and Jensen [7].

The melting point was determined on a micro melting-point apparatus, UV spectra was measured using a UV-Vis Shimadzu spectrometer in MeOH, and 1 H and 13 C NMR spectra were recorder on a JEOL J-500 spectrometer operating at 500 MHz (1 H) and 125 MHz (13 C) using TMS as an internal standard. Optical rotatory was measured using a PerkinElmer 341 polarimeter. Gravitation column chromatography (GCC) was carried out using Merck Si gel 60 (70–200 mesh), vacuum liquid chromatography (VLC) and radial chromatography using Merck Si gel 60 GF $_{254}$, and thin-layer chromatography (TLC) using procoated Si gel plates (Merck kieselgel 60 F $_{254}$ 0.25 mm). Solvents used in this research were of analytical and technical grade and were distilled before use.

The tree bark of *D. oblongifolia* Dyer was obtained from Gunung Mali, Tempunak, Sintang, West Kalimantan, in December 2012. The specimen was identified at Biological Research Center, LIPI, Bogor, Indonesia, and a voucher specimen has been deposited at the herbarium.

The powdered tree bark of *Dryobalanops oblongifolia* (5 kg) was macerated with acetone for 2×24 h and then concentrated under reduced pressure to give a gummy brownish extract. The extract was divided into acetone–diethyl ether soluble and insoluble fractions. The acetone-diethyl ether soluble fraction (48 g) was fractionated using VLC (n-hexane–ethyl acetate, increasing polarity) to give four major fractions A–D. From TLC analysis, fraction D (3.2 g) was chosen for further analysis. Fraction D was GCC refractionated repeatedly using n-hexane–ethyl acetate 5:5 – ethyl acetate 100% to yield fractions D1–D3. Fraction D1, on repeated purification using radial chromatography with chloroform–methanol (9:1), yielded compound 1 (14.8 mg).

Compound 1 was obtained as an amorphous brown solid, mp 220–222°C (dec), $[\alpha]_D^{25}$ –2°. The molecular formula, $C_{28}H_{22}O_6$, of compound 1 was established based on HR-FAB-MS (m/z 454.1416 [M]⁺), corresponding to a resveratrol dimer.

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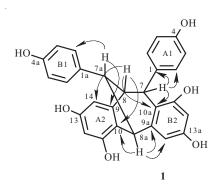


Fig. 1. Structure of compound **1** and HMBC correlations.

The UV spectra of compound 1 showed absorption at 220 and 282 nm, typical for oligomeric stilbene chromophores. The ¹³C NMR spectra of compound 1 showed 22 distinct signals, which represented 28 atoms of carbon. The ¹H NMR spectra of compound 1 exhibited four signals of an *ortho*-coupled aromatic hydrogen at δ 7.02, 6.72, 6.69, and 6.50 (each 2H, d), indicating the presence of two units of 4-hydroxyphenyl. Four signals of a *meta*-coupled aromatic hydrogen at δ 6.38, 6.36, 6.08, and 6.01 (each 1H, d) indicated the presence of two units of 1,2-substituted-3,5-dihydroxyphenyl. Four signals of an aliphatic methane hydrogen singlet appeared at δ 4.08, 4.03, 3.56, and 3.23. The chemical shift values of the four aliphatic methine hydrogens indicated that this structure did not form an oxygen heterocyclic ring. The four methine hydrogens appearing as a singlet signal showed that all the dihedral angles of the vicinal hydrogen approach 90°, so that the small values of the vicinal coupling constants prevented the four methine hydrogens did not undergo splitting [8, 9]. The relation of inter-units shown in compound 1 was confirmed by analysis of ¹H-¹³C long-range couplings on the HMBC spectrum (Fig. 1). The HMBC spectrum of compound 1 showed some correlations between H-7/C-8, H-7/C-10a, H-8/C-10a, H-8/C-10a, H-8a/C-10a, H-8a/C-10, and H-8a/C-9a; it showed that rings A₂ and B₂ were connected by C-7, C-8, and C-8a. Moreover, the correlations between H-7a/C-9, H-8a/C-8, H-8/C-8a, H-8/C-10a, and H-7/C-7a proved that C-7a formed a bicyclo ring between C-8 and C-8a. The aliphatic methine hydrogens at δ 3.56, 4.03, and 4.08 were long-range coupled with the aromatic hydrogens at δ 6.69, 6.36, and 7.02, respectively. In addition, the methine hydrogen at δ 3.23 with another methine hydrogen at δ 4.03 displayed w-relationships of the proton system [8, 9].

Based on the analysis of NMR and 2D NMR and comparation of the NMR spectroscopic data between compound 1 and the published data of (+)-ampelopsin F that was isolated from *Ampelopsis brevipedunculata* [9], it was concluded that this compound has the (-)-ampelopsin F structure.

(-)-Ampelopsin F (1), amorphous brown solid, mp 220–222°C (dec); $[\alpha]_D^{25}$ –2° (c 0.1; MeOH). UV spectrum (MeOH, λ_{max} , nm): 220 and 282. HR-FAB-MS m/z [M]⁺ 454.1416 (calcd for $C_{28}H_{22}O_6$, 454.1410). ¹H NMR (500 MHz, CD_3OD , δ , ppm, J/Hz): 7.02 (2H, d, J = 8.6, H-2, 6), 6.72 (2H, d, J = 6.9, H-3, 5), 6.69 (2H, d, J = 8.3, H-2a, 6a), 6.50 (2H, d, J = 6.7, H-3a, 5a), 6.38 (1H, d, J = 2, H-14), 6.36 (1H, d, J = 2.3, H-14a), 6.08 (1H, d, J = 2.6, H-12a), 6.01 (1H, d, J = 2, H-12), 4.08 (1H, s, H-7), 3.56 (1H, s, H-7a), and 3.23 (1H, s, H-8). ¹³C NMR (125 MHz, CD_3OD , δ , ppm): 158.5 (C-13), 158.1 (C-11a), 157.2 (C-13a), 156.1 (C-4, 4a), 153.2 (C-11), 147.9 (C-9a), 147.5 (C-9), 139.1 (C-1), 136.1 (C-1a), 130.1 (C-2, 6), 129.4 (C-2a, 6a), 128.7 (C-10), 115.8 (C-3, 5), 115.6 (C-3a, 5a), 114.2 (C-10a), 105.8 (C-14a), 104.4 (C-14), 102.0 (C-12, 12a), 59.3 (C-8), 50.9 (C-7a), 49.9 (C-8a), 47.6 (C-7).

The antimalarial activity of compound 1, expressed as IC_{50} , is the sample concentration required to inhibit parasite growth by 50%. The antimalarial activity was tested by introducing a series of samples with different concentrations containing complete medium (RPMI 1640 medium with 10% serum O) + erythrocyte into microwell plates and then adding a suspension of *Plasmodium falciparum* (chloroquine sensitive strain, 3D7) [10]. Pyrimethamine was used as positive control. The percent average parasitemia and the percent average inhibition of parasite growth were determined by counting the amount of infected erythrocytes in every 1000 erythrocytes. The value of IC_{50} was obtained by analyzing the data of the antimalarial activity test result using probit analysis of the SPSS program.

The antimalarial activity test of (–)-ampelopsin F against P. falciparum gave an IC_{50} value of 0.001 µg/mL (with pyrimethamine as a positive control, IC_{50} 0.03 µg/mL). This result showed that (–)-ampelopsin F has promise as an antimalarial agent.

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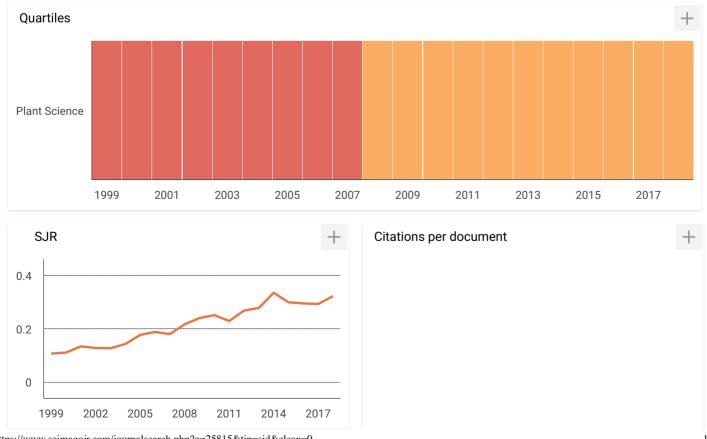
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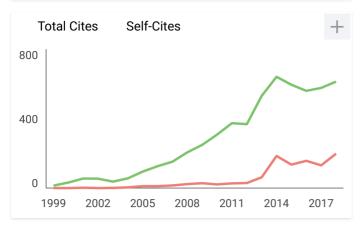
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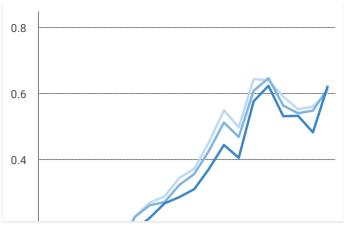
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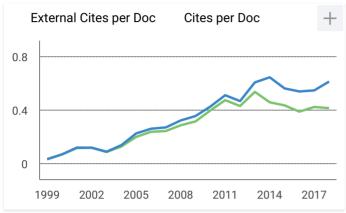
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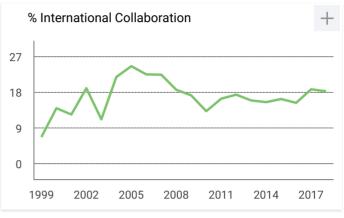


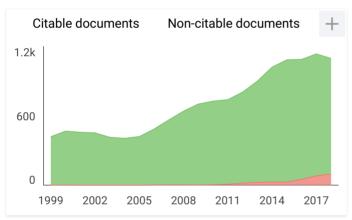


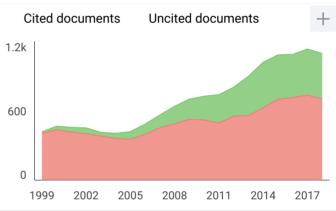


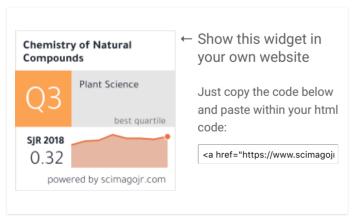










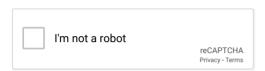


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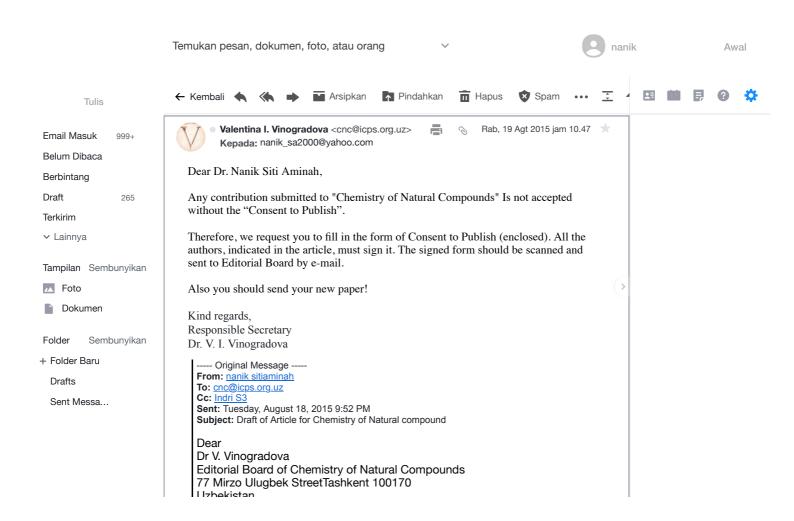
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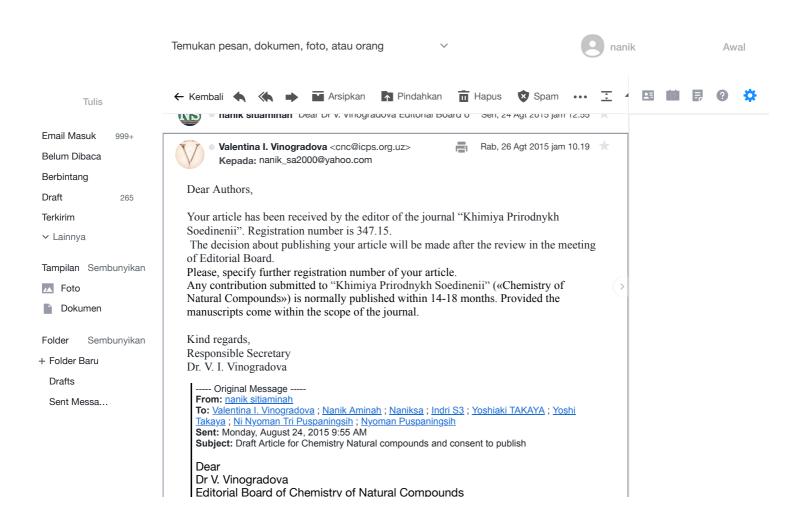
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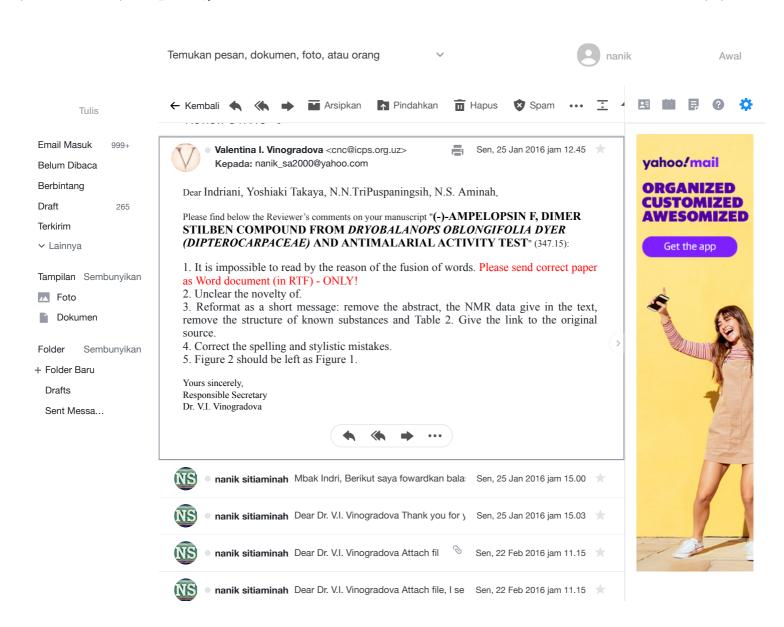
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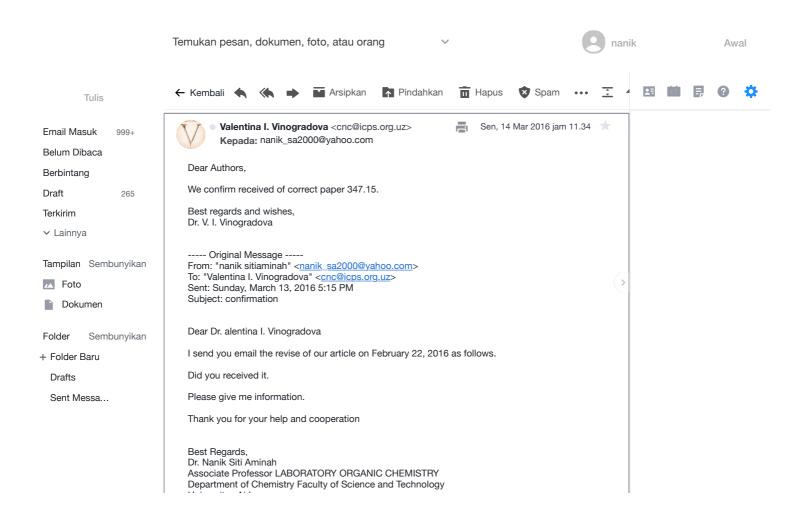
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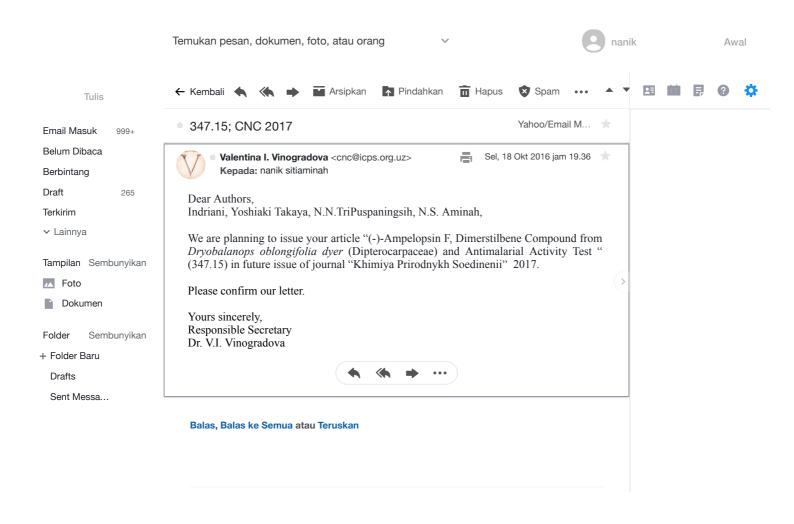
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