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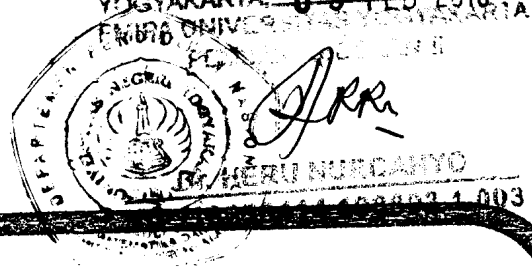
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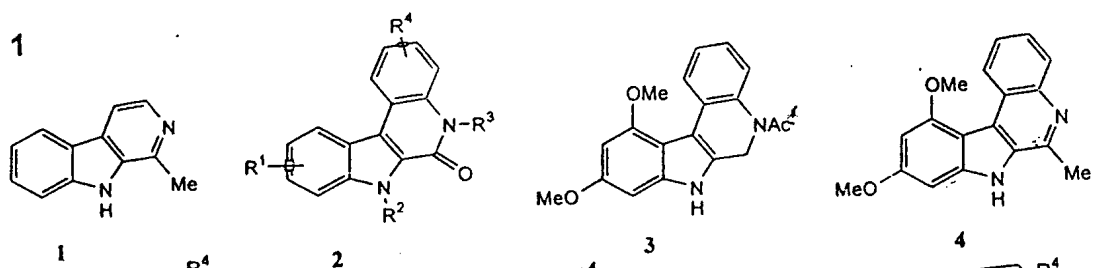
# Indonesian Journal of Chemistry

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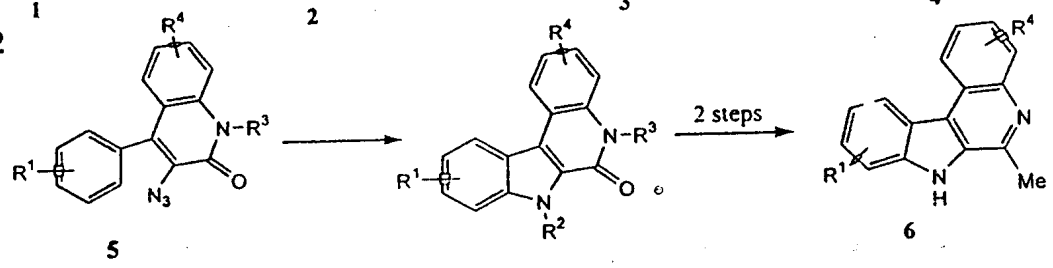
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TELAH DIPERIKSA KESEBENARANNYA  
DAN SESUAI DENGAN ASLINYA  
YOGYAKARTA, 09 FEB 2010  
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Scheme 1



Scheme 2



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## STRUCTURE IDENTIFICATION OF A TRIMER STILBENOID COMPOUND FROM STEM BARK *Hopea nigra* (DIPTEROCARPACEAE)

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### ABSTRACT

Bioactivity as antihepatotoxic directed fractionation of acetone extract from the stem bark of *Hopea nigra* (Dipterocarpaceae) afforded a stilbenoid trimer, namely vaticanol G (1). The structure of this compound was elucidated based on physical and spectroscopic data (UV, IR, MS, <sup>1</sup>H and <sup>13</sup>C NMR 1D and 2D).

Keywords: Vaticanol G; *Hopea nigra*; antihepatotoxic; Dipterocarpaceae.

### INTRODUCTION

Dipterocarpaceae is one of the largest families found in the tropical forest Indonesia. The plants are distributed from the west of Indonesia until Papua (Irian Jaya) and the mostly in Kalimantan, there for the timber of these plants are usually called "meranti" or "Kayu Kalimantan". Dipterocarpaceae consists of about 16 genus and 600 species [1,2] and until now only few species have been investigated.

Some chemical constituents that can be found from this plants include arylpropanoid, benzofuran, flavanoid, polyphenol, resveratrol oligomers and terpenoid [3-16]. The resveratrol oligomer from Dipterocarpaceae plants have various structure from simple structure as it monomer, dimer, trimer, until hexamer [3-14]. These structures are very interesting and showed interesting biological activity, such as antibacterial, anticancer, antihepatotoxic, and anti-HIV [3-17]. Thus Dipterocarpaceae plants are very potential for chemical research in natural product and pharmaceutical industry.

This paper deals with the isolation and structure elucidation of vaticanol G (1) from the stem bark *H. nigra*. The compound from polar fraction acetone extract showed as antihepatotoxic was conducted by *in vivo* using the white rats that induced by CCl<sub>4</sub>. The structure of this compound based on the analysis spectrum of UV, IR, MS and NMR included 1D and 2D NMR (<sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HMBC and NOESY).

### EXPERIMENTAL SECTION

#### General Experimental Procedure

UV and IR spectra were measured with varian Cary 100 Conc dan Shimadzu 8300 FTIR respectively. <sup>1</sup>H dan <sup>13</sup>C NMR spectra were recorded with Jeol JNM A-5000 spectrophotometers, operating at 600.0 MHz (<sup>1</sup>H) dan 150.0 MHz (<sup>13</sup>C) using residual and deuterated solvent peaks as internal standards. MS spectra were obtained with a JMS-AM 20 spectrometer, using the mode FAB. Vacuum liquid chromatography (VLC) was carried out using Merck Si gel Merk 60 GF<sub>254</sub> (230-400 mesh), column chromatography using Si-gel Merk 60 (200-400 mesh), and TLC analysis on precoated Si gel plates Si-gel Merk Kieselgel 60 F<sub>254</sub> 0.25 mm, 20 x 20 cm.

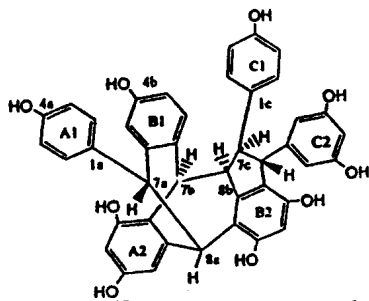
#### Plant Material

Samples of the stem bark of *H. nigra* were collected in Desember 2003 from the Experimental Garden in Carita, Banten, Indonesia. The plant was identified by the staff at the Herbarium Bogoriense, Kebun Raya Bogor, Bogor, and a voucher specimen had been deposited at the Herbarium.

#### Extraction and Isolation

The dried and milled stem bark of *H. nigra* (4.6 kg) was extracted three times exhaustively with Me<sub>2</sub>CO at room temperature, each time by soaking in 5 L of solvent overnight before it was decanted. The combined extracts was evaporated under reduced pressure to give a brown gum (374 g). A portion (40 g) was then subjected to fractionated by VLC (silica gel GF 60 Merk 250 g; φ: 10 cm, t = 10 cm), using n-hexane, n-hexane-EtOAc, EtOAc, Me<sub>2</sub>CO, and MeOH of increasing polarity as eluents

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Table 1 <sup>1</sup>H and <sup>13</sup>C NMR data of vaticanol G (1)

No	$\delta_H$ (multiplicity, J in Hz)	$\delta_C$	HMBC (H-C)
1a	-	139.8	-
2a,6a	6.45 (br s)	130.1	C-8a, C-7a, C-1a, C-4a
3a,5a	6.46 (br s)	114.6	C-8a, C-7a, C-1a, C-4a
4a (OH)	7.89 (br s)	155.4	C-3a,5a, C-4a
7a	4.55 (d, 4.3)	57.1	C-10b, C-3b, C-1b, C-2a,6a, C-1a, C-9a, C-8a
8a	4.63 (d, 4.3)	50.2	C-14a, C-10b, C-10a, C-1a, C-9a, C-7a, C-9b,
9a	-	141.8	-
10a	-	125.9	-
11a (OH)	8.01 (br s)	153.1	C-10a, C-12a, C-11a
12a	6.20 (d, 2.8)	101.6	C-14a, C-10a, C-11a, C-13a
13a (OH)	7.59 (br s)	155.8	C-12a, C-14a, C-13a
14a	5.67 (d, 2.8)	111.4	C-12a, C-10a, C-13a
1b	-	129.1	-
2b	-	141.6	-
3b	6.07 (d, 2.6)	119.7	C-5b, C-1b, C-4b, C-7a
4b (OH)	7.40 (br s)	154.8	C-3b, C-4b
5b	5.77 (dd, 8.4; 2.6)	112.7	C-1b, C-3b, C-6b
6b	6.02 (d, 8.4)	134.9	C-2b, C-4b, C-1b, C-7b
7b	4.89 (d, 3.0)	42.6	C-10a, C-2b, C-6b, C-9a, C-8b, C-7c, C-9b,
8b	3.85 (dd, 8.9; 3.0)	53.8	C-10b, C-10a, C-1b, C-1c, C-7b, C-7c, C-9b
9b	-	146.9	-
10b	-	117.5	-
11b (OH)	8.48 (br s)	154.9	C-10b, C-12b, C-11b
12b	6.46 (s)	101.8	C-10b, C-14b, C-13b, C-11b
13b (OH)	7.59 (br s)	152.8	C-12b, C-14b
14b	-	121.8	-
1c	-	136.9	-
2c	5.92 (br s)	127.7	-
3c	5.98 (br s)	114.6	-
4c (OH)	7.85 (br s)	156.4	C-5c, C-4c,

to give twenty fractions. These fractions were combined to give two major fractions A (7.8 g) and B (7.4 g). Fraction B (7.4 g) was found to be significant as antihepatotoxic activity and then was repeatedly separated and purified by column chromatography to give vaticanol G (1) 200 mg.

Vaticanol G (1) was obtained as a brown powder, m.p. 240 °C, UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ): 208 (5.95); 234 (sh) (5.72); 280 (5.16), IR (KBr)  $\nu_{max}$ : 3296; 1609; 1510; 1445; 1243; 1142; 1012; 833  $cm^{-1}$ , <sup>1</sup>H and <sup>13</sup>C NMR (Me<sub>2</sub>CO-d<sub>6</sub>, 600.0 and 150 MHz) see Table 1. FABMS  $m/z$  680 [M<sup>+</sup>] (C<sub>42</sub>H<sub>32</sub>O<sub>9</sub>).

## RESULT AND DISCUSSION

From the acetone extract of stem bark *H. nigra*, after separated and repeatedly purification by extensive chromatography resulted the compound 1. Vaticanol G (1) was obtained as a brown powder, m.p. 240 °C. Its UV spectrum showed absorption maxima at 280 nm, suggesting the presence of unconjugated phenolic chromophore. The IR spectrum exhibited hydroxyl group (3296  $cm^{-1}$ ), C=C aromatic (1609; 1510; 1445  $cm^{-1}$ ), and monosubstituted benzene (833  $cm^{-1}$ ), these spectra characteristic absorptions for supporting 1 to be an oligostilbene. The positive ion FABMS exhibited an [M]<sup>+</sup> ion at  $m/z$  680, together with NMR data, corresponding to a molecular formula C<sub>42</sub>H<sub>32</sub>O<sub>9</sub>, for a resveratrol trimer.

The <sup>1</sup>H NMR spectrum of 1 in d<sub>6</sub>-acetone exhibited signals for two sets of 4-hydroxybenzene at  $\delta$  6.45 (br s) and 6.46 (br s), each 2H, at  $\delta$  7.13 (br s); 6.67 (br s); 5.98 (br s); and 5.92 (br s), each 1H (ring of A1 and C1), and one unit 1,2,4-trisubstituted benzene at  $\delta$  6.07 (1H, d, J = 2.6 Hz); 6.02 (1H, d, J = 10.6 Hz). Additionally, the <sup>1</sup>H NMR spectrum exhibited signals for a set of aromatic signals at  $\delta$  5.77 (1H, dd, J = 8.4; 2.6 Hz) (ring B1), one unit 1,3,5-trisubstituted benzene at  $\delta$  6.12 (1H, t, J = 2.6; 2.6 Hz) and 5.96 (2H, d, J = 2.6 Hz) (ring C2), one unit 1,2,3,5-tetrasubstituted benzene at  $\delta$  6.20 (1H, d, J = 2.8 Hz) and 5.67 (1H, d, J = 2.8 Hz) (unit A2), and one unit 1,2,6-trisubstituted-3,5-dihydroxybenzene ( $\delta$  6.46 (s), (ring B2). The six substituted benzene ring suggesting with 24 DBE (Double Bond Equivalent). Beside that, the <sup>1</sup>H NMR spectrum exhibited two signals aliphatic proton which correlated at <sup>1</sup>H-<sup>1</sup>H COSY spectrum, characteristic with unit of -CH-CH- [ $\delta$  4.63 (1H, d, J = 4.3 Hz) and 4.55 (1H, d, J = 4.3 Hz) (unit D), and four signals assignable to two-coupled aliphatic protons characteristic with unit of -CH-CH-CH-CH- [ $\delta$  4.89 (1H, d, J = 3.0 Hz); 3.85 (1H, dd, J = 8.9; 3.0 Hz); 3.51 (1H, d, J = 8.9 Hz); and 4.11 (1H, s) (unit E)].

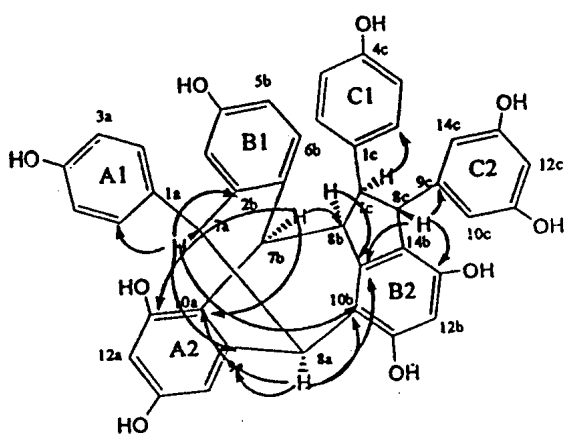


Fig 1 Significant HMBC (H→C) correlation of 1

The characteristic of aliphatic protons signal was not indicated for trans-2,3-diaryl-dihydrobenzofuran moiety, and support for structure 1 was a trimeric resveratrol with aliphatic trisiclo skeleton, were similar to those of vaticanol G isolated from *Vatica rassak* [8]. Complete assignment of all proton - bearing carbon signals were made possible by analysis of the HMQC spectrum, and support for structure 1 was obtained from significant cross-peak in HMBC measurement.

The NMR 2-D HMQC and HMBC spectrum of 1 showed correlation HMBC (Fig. 1) proton at  $\delta$  4.55 (H-7a) with carbon signals at  $\delta$  141.6 (C-2b); 141.8 (C-9a); and 117.5 (C-10b), and proton at  $\delta$  4.89 (H-7b) showed long range correlations with carbon at  $\delta$  125.9 (C-10a) and 53.8 (C-8b). As well as proton at  $\delta$  4.63 (H-8a) showed long range correlations with carbon at  $\delta$  141.8 (C-9a) and 117.5 (C-10b). Furthermore, the methylene proton at  $\delta$  4.11 (H-8c) showed long range correlations with carbon at  $\delta$  152.8 (C-13b) and 106.3 (C-10c/14c), and the latter proton at  $\delta$  3.51 (H-7c) correlation with carbon at  $\delta$  127.7 (C-2c) and 130.3 (C-6c). These spectroscopic evidence were in agreement with the proposed structure 1 for vaticanol G [8].

The relative configuration of 1 (Fig. 2) was also established by NOESY experiments. The presence of NOESY between OH-11b/H-8a; H-8a/H-2a,6a indicated that proton H-7a and H-8a has *trans* orientation. As well as correlation NOE between proton H-7b / H-8b, H-7b/H-7c, H-8c/H-10c, and H-8c/H-2c respectively supporting that proton H-7b/H-8b and H-8b/H-7c, has *cis* orientation, H-7c/H-8c *trans* orientation. Therefore, the relative configuration from the chiral carbons appropriate with vaticanol G (1) which previous reported from *Vatica rassak* [8]. Vaticanol-G (1) as a stilbenoid trimer with tribenzobisiclo [3,3,2]dekatriena skeleton, is a rare structure of natural products.

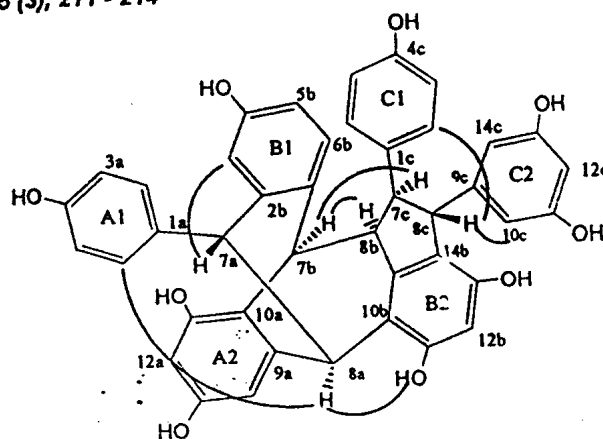


Fig 2 Significant NOESY correlation of 1

## CONCLUSION

From the polar fraction extract acetone stem bark of *H. nigra* can be isolated a trimer stilbenoid, vaticanol G (1).

## ACKNOWLEDGEMENT

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