

**A TRIMER OLIGOSTILBENOID FROM INDONESIAN  
*VATICA PAUCIFLORA* BLUME (DIPTEROCARPACEAE)**

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Abstract

A trimer oligostilbenoid, named Stenophyllol B has been isolated from the ethyl acetate extract of the stem bark of *Vatica pauciflora* Blume. The structure was elucidated based on spectroscopic data including UV, IR, MS, NMR ( <sup>1</sup>H & <sup>13</sup>C ), HMQC, HMBC, and COSY. This compound showed medium activity ( IC : 8,5 µl/ml) with P388 murine leucaemia cell. The isolation and structure elucidation of this compound will be described.

Key words : Stenophyllol, *Vatica pauciflora* Blume, Trimer oligostilbenoid

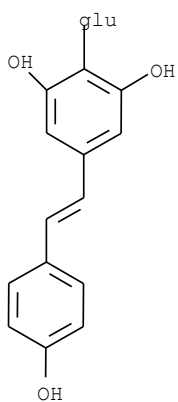
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## Introduction

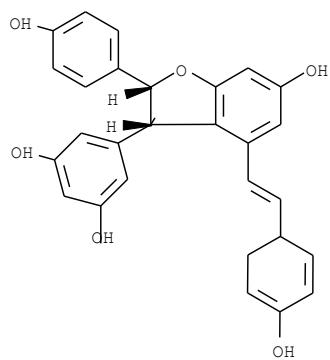
Dipterocarpaceae is one of the largest families found in the tropical forest Indonesia. The plants are distributed from the west of Indonesia until Papua (Irian Jaya) and the mostly in Kalimantan, there fore the timber of these plants are usually called “meranti” or “ Kayu Kalimantan”. Dipterocarpaceae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

Some chemical constituents that can be found from this plants include arilpropanoid, benzofuran, flavanoid, polyphenol, oligostilbenoids, and terpenoid<sup>2,3</sup>. The oligostilbenoids from Dipterocarpaceae plants have various structure from simple structure as like resveratrol monomer, resveratrol dimer, resveratrol trimer, until resveratrol hexamer<sup>4</sup>. These structure are very interesting and showed interesting biological activity, such as antibacterial, anticancer, antihepatotoxic, and anti-HIV. Thus Dipterocarpaceae plants are very potential for chemical research in natural product and pharmaceutical industry. Recently a number of novel had been reported such as resveratrol-12C- $\beta$ -glucopyranoside (**1**) from *Shorea hemsleyana*<sup>5</sup>,  $\epsilon$ -viniferin (**2**) from *V. affinis*<sup>6</sup>,  $\alpha$ -viniferin (**3**) from *S. hemsleyana*<sup>5</sup>, hopeaphenol (**5**) from *S. hemsleyana*<sup>5</sup>, and vaticanol D (**5**) from *V. rassak*<sup>4</sup>.

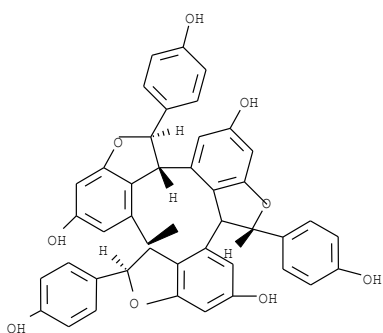
The aims of this research is to isolate, determine structure and biological activity of these compounds from *V. pauciflora*. Until now these species never have been investigated by other researches. Thus in the present work we report on the isolation, structure determination, and biological activity of a trimer oligostilbenoid. Stenophyllol B (**6**) obtained from the fraction ethyl acetate. The biological activity of these compound were evaluated against P388 murine leucaemia cells.



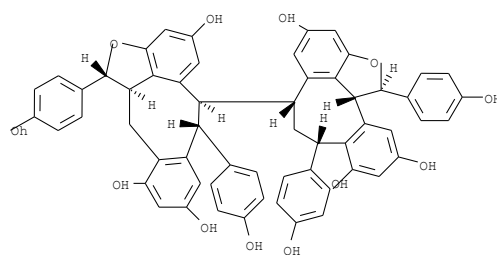
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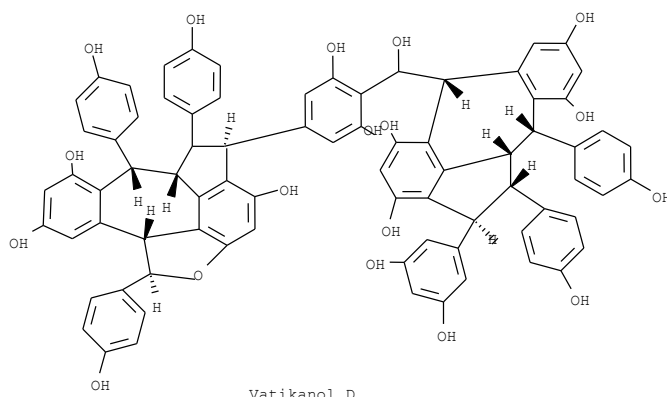
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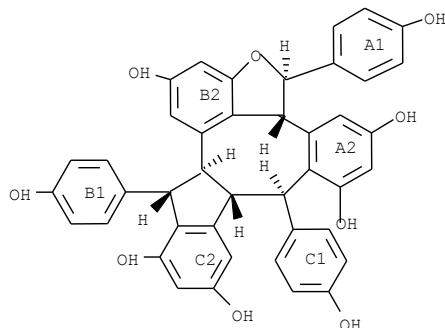
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(4)



(5)



6

## Experimental Section

### General Experimental Procedures

Melting points were determined on a micro melting point apparatus and are uncorrected. Optical rotation was measured in MeOH on a JASCO DIP-370 digital polarimeter. UV and IR spectra were obtained using varian Cary 100 Conc and one Perkin Elmer Spectrophotometers.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with JNM A 6000 spectrometer operating at 500 MHz ( $^1\text{H}$ ) and 500 MHz ( $^{13}\text{C}$ ) using TMS as an internal standart. FAB-MS were obtained with Jeol JMS- AM 20 mass spectrometer using the EI mode. VLC was carried out using Merk Si-gel 60 GF<sub>254</sub>, flash chromatography with Merk Si-gel 60 (230 – 400 mesh), and TLC analysis on precoated Si-gel plates (Merk kieselgel 60 F<sub>254</sub> 0,25 mm ).

### Plant Material

Sample of the steam bark of *V. pauciflora* were collected in September 2000 from the research garden Dramaga Bogor , Indonesia. The plant was identified by the staff at the Herbarium Bogorienses, Bogor, Indonesia.

## Bioactivity Test

The sitotoxicity of the compound was evaluated with P388 Murine leukaemia cells.

## Extraction and Isolation

The milled dried steam bark (5 Kg) was extracted exhaustively with MeOH ( 3 x 15 L) for 24 hours. The combined extracts were evaporated under reduced pressure to small volume. The extract was fractionated with hexane ( 3 x 500 ml), methylene chloride ( 3 x 500 ml) and then with ethyl acetate. A part of the ethyl acetate fraction (35 gr) was preabsorbed on silica gel and chromatographed over a column of silica gel with amounts of hexane in ethyl acetate as eluent with increased gradient polarity. Fraction which gave the same Rf on TLC were combined and rechromatographed in the same way. The less polar fraction yielded material which was purified by repeated chromatography to give S-1 (**6**) as amorf pale yellow (42 mg). Stenophyllol B was obtained as amorf pale yellow, m.p 230 °C (decompous),  $[\alpha]_D$  : -20 (in 0,1 MeOH), UV (MeOH)  $\lambda$  max : 205; 230 (sh); 285 nm, IR (KBr)  $\nu$  max : 3393 (OH); 1613; 1462; (C=C aromatik); 1337; 1231;1171; 1124; and 831  $\text{Cm}^{-1}$ .  $^1\text{H}$ - NMR (  $\text{AC}_2\text{O}$ , 500 MHZ) and  $^{13}\text{C}$  – NMR see Table 1. FAB-MS m/z (relative intensity)  $[\text{M}^+ + 1]^+$  : 680,89 (84); 613,35 (20); 573,38 (20); 466,37 (100); and 391 (36).

## Discussion

The FABMS of stenophyllol B (**6**) gave an  $[M]^+$  ion at  $m/z$  680 consistent with a molecular formula of  $C_{42}H_{32}O_9$  and is regarded as a stilbene trimer. The IR spectrum of compound **6** showed absorptions broads typical of hydroxyl ( $3393\text{ cm}^{-1}$ ), aromatic ring ( $1613$ ;  $1462$ ;  $1337\text{ cm}^{-1}$ ) and a prominent band at  $831\text{ cm}^{-1}$  indicative of 1, 4 disubstituted aromatic ring. The predicted structure of **6** was further confirmed by the NMR data ( $^1\text{H}$  and  $^{13}\text{C}$ ) (Table 1) including HMQC, H-H COSY, and HMBC and compared with those reported in literature <sup>7</sup>.

It can be seen from the NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) spectral data of **6** (Table 1). The  $^1\text{H}$  – NMR spectrum showed the presence of three sets of NMR coupled aromatic protons assignable to three 4 – hydroxyphenyl groups [ $\delta$  6,89 (2 H, d,  $J$  : 8,5 Hz, H 2a, 6a); 6,75 (2 H, d,  $J$  : 8,5 Hz, H 3a, 5a); 7,22 (2H, d,  $J$  : 8,5 Hz, H 2c, 6c); 6,70 (2 H, d, 8,9, H 3c, 5c)], three sets of proton assignable to 1,2,3,5-tetra substituted benzene rings [ $\delta$  6,33 (1 H, d,  $J$  : 2,2 Hz, H 12a); 6,26 (1 H, d, 2,2 Hz, H 14a); 6,23 (1H, d 1,6 Hz, H 12b); 6,79 (1 H, br s), H 14b); 6,08 (1 H, m, H 12c and 14 c), a set mutually coupled aliphatic methines [ $\delta$  5,85 (1 H, d,  $J$  : 4,0 Hz, H 7a); 5,09 (1 H, d,  $J$  : 3,9 Hz, H 8a)], a sequence of aliphatic methine protons coupled succesively in this order [ $\delta$  4,75 (1 H, d,  $J$ : 6,6, H 7b), 3,44 (1 H, dd,  $J$  : 6,5; 7,0 Hz, H 8b); 4,34 (1H dd,  $J$  : 9,5; 7,8 Hz, H 8c); 5,38 (1H, d,  $J$  : 9,9, H 7c)], in addition to eight phenolic hydroxyl groups ( $\delta$  7,9; 8,6).

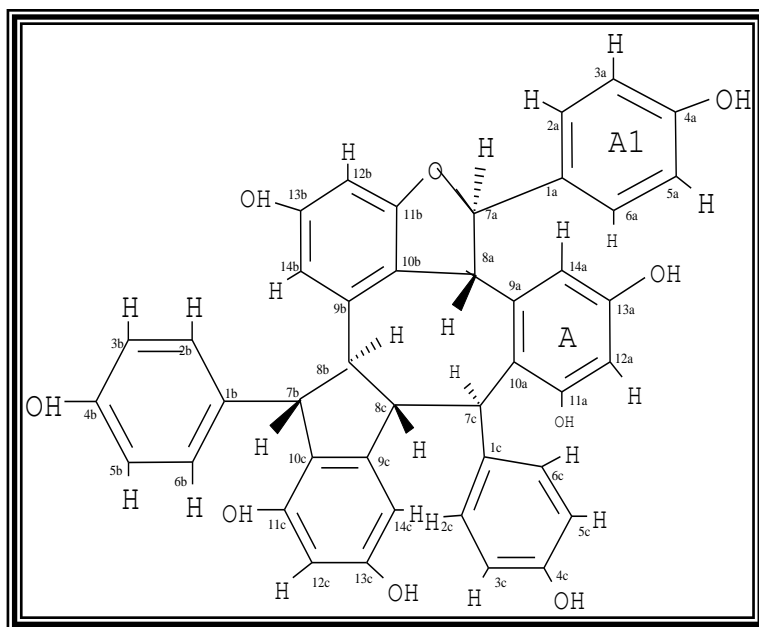
Table 1. The NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) spectral data of compound **6** and stenophyllol B

No	Compound <b>6</b>		Stenophyllol B	
	$\delta$ H (Ac <sub>2</sub> O 500 MHz)	$\delta$ C (Ac <sub>2</sub> O, 500 MHz)	$\delta$ H (Ac <sub>2</sub> O 500 MHz)	$\delta$ C (Ac <sub>2</sub> O, 500 MHz)
1a	-	134,4	-	134,6
2a,6a	6,89 (d 8,5)	127,9	6,89 (d 8,8)	127,2
3a,5a	6,75 (d 8,5)	115,8	6,76 (d 8,8)	116,0
4a	-	157,5	-	157,6
7a	5,85 (d 4,0)	87,9	5,84 (d 3,9)	88,0
8a	5,09 (d 3,9)	52,4	5,09 (d 3,9)	52,5
9a	-	141,2	-	141,4
10a	-	123,3	-	123,4
11a	-	156,3	-	156,5
12a	6,33 (d 2,2)	104,8	6,26 (d 1,9)	101,3
13a	-	157,9	-	158,1
14a	6,26 (d 2,2)	104,8	6,26 (d 1,9)	105,0
1b	-	139,4	-	136,9
2b,6b	7,22 (d 8,4)	129,9	7,21(d 8,5)	130,0
3b,5b	6,68 (d 8,7)	115,7	6,68 (d 8,5)	115,8
4b	-	155,9	-	156,1
7b	4,75 (d 6,6)	51,7	4,73 (d 6,3)	51,9
8b	3,44 (dd 6,5;7,0)	56,2	3,43 (brd 6,3)	56,4
9b	-	143,9	-	144,1
10b	-	120,4	-	120,6
11b	-	160,2	-	160,4
12b	6,23 (d 1,6)	95,8	6,24 (d 1,9)	95,9
13b	-	158,6	-	158,7
14b	6,79 (br s)	106,7	6,80 (br s)	106,8
1c	-	136,7	-	139,5
2c,6c	7,31 (d 8,5)	129,8	7,31 (d 8,5)	129,9
3c,5c	6,70 (d 8,9)	115,7	6,70 (d 8,5)	115,8
4c	-	156,0	-	156,2
7c	5,38 (d 9,9)	47,1	5,37 (d 9,8)	42,2
8c	4,34 (dd 9,5; 7,8)	53,4	4,34 (dd 9,8; 7,8)	53,5
9c	-	150,6	-	150,8
10c	-	123,3	-	123,4
11c	-	154,5	-	154,6
12c	6,08 (m)	102,2	6,08 (m)	102,3
13c	-	158,9	-	154,6
14c	6,08(m)	102,9	6,08 (m)	103,1

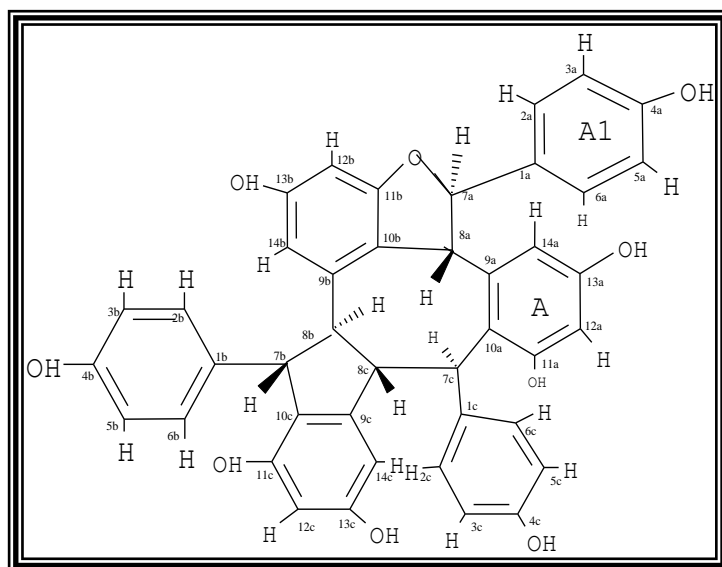
All protonated carbon signals in the  $^{13}\text{C}$ -NMR spectrum were assigned completely by the HMQC spectrum and are listed in Table 1. The  $^{13}\text{C}$ - NMR spectrum of **6** exhibited forty two signals corresponded to six methine alifatic carbon at [ $\delta$  47,1; 51,7; 52,4; 53,4; 56,2; 87,9], eighteen methine aromatic carbon at [ $\delta$  2 x 127,9; 2 x 115,8; 101,2; 104,8; 2 x 129,9; 2 x 115,7; 95,8; 106,7; 2 x 129,8; 2 x 115,7; 102,2, 102,9] and nine quaternary carbon at [ $\delta$  134,4; 141,2; 123,3; 139,4; 143,9; 120,4; 136,7; 150,6; 123,3], lowfield signals at [ $\delta$  157,5; 156,3; 157,9; 155,9; 160,2; 158,6; 156,0; 154,5; 158,9 ] were assigned to nine oxyaryl carbons.

Assignment for all the functional groups in compound **6** we achieved by heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiment. In the HMBC spectrum of **6** showed  $^{13}\text{C} - ^1\text{H}$  long range correlations were observed between C-2a(6a) / H - 7a, C-8a/ H-14a; C-9b/ H-8a; C-9b/ H-14 b; and C-7c/ H-2c(6c); C-8c / H-7b; C-7b/ H-2b(6b) (Fig. 1). In the  $^1\text{H} - ^1\text{H}$  long range COSY spectrum, the mutually coupled methine protons ( H-7a and H-8a) were correlated with H-2a (6a) and H-14a respectively. The long range correlation were observed to between H-7c / H-8c; H-8c/ H-8b; H-7b / H-2b(6b) (fig 2). These result indicated that a resveratrol unit formed a 2,3- aryldihydrobenzofuran skeleton through ring B2. These spectroscopic evidence were in agreement with the proposed structure assigned to stenophyllol B (**6**) came from comparison of the NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) to those reported in the literatures <sup>7</sup>.





**Fig. Significant HMBC Correlation of Stenophyllol B**



**Fig 2. Significant H-H Cosy Correlations of Stenophyllol B**

These compound showed medium activity ( IC 8,5  $\mu\text{g/ml}$ ) with P388 murine leuceimia cells. A trimer oligostilbenoids type have been isolated from several other Dipterocarpaceae and Leguminace species<sup>7,8,9</sup>.

### **Acknowledgment**

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